UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENICAS QUÍMICAS
Departamento de Química Orgánica

TESIS DOCTORAL

LPA_1 and LPA_2 receptors as new therapeutic targets for the treatment of central nervous system pathologies

Los receptores LPA_1 y LPA_2, como nuevas dianas terapéuticas para el tratamiento de patologías del sistema nervioso central

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Debora Zian

Directores

Mª Luz López Rodríguez
Silvia Ortega Gutiérrez
Henar Vázquez Villa

Madrid, 2018

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE CIENCIAS QUÍMICAS

Departamento de Química Orgánica I

LPA_1 AND LPA_2 RECEPTORS AS NEW THERAPEUTIC TARGETS FOR THE TREATMENT OF CENTRAL NERVOUS SYSTEM PATHOLOGIES

LOS RECEPTORES LPA_1 Y LPA_2 COMO NUEVAS DIANAS TERAPÉUTICAS PARA EL TRATAMIENTO DE PATOLOGIAS DEL SISTEMA NERVIOSO CENTRAL

PhD candidate

Debora Zian

Advisors:
Dra. Mª Luz López Rodríguez
Dra. Silvia Ortega Gutiérrez
Dra. Henar Vázquez Villa

MADRID, 2017

A mi familia

El presente trabajo ha sido realizado en el Laboratorio de Química Médica del Departamento de Química Orgánica I de la Facultad de Ciencias Químicas de la Universidad Complutense de Madrid, bajo la supervisión de la **Catedrática Dra. María Luz López Rodríguez** y de las **Dras. Silvia Ortega Gutiérrez** y **Henar Vázquez Villa**, a quienes deseo expresar mi más sincero agradecimiento por su calurosa acogida en este grupo de investigación, por sus continuas enseñanzas a lo largo de estos años, y muy especialmente, por todo el ánimo, apoyo y confianza depositados en este proyecto y en mí.

Asimismo, quiero expresar mi agradecimiento:

Al Prof. Jerold Chun de The Scripps Research Institute por su acogida en el laboratorio y sus enseñanzas en la técnica de *Backscattering Inteferometry*.

Al Prof. Antonio Ferrer Montiel de la Universidad Miguel Hernández de Elche, en cuyo laboratorio se han llevado a cabo los experimentos de desensibilización neuronal.

Al Prof. Fernando Rodríguez de Fonseca del Instituto de Investigación Biomédica de Málaga, en cuyo laboratorio se están llevando a cabo los experimentos in vivo de dolor neuropático.

Al Prof. Rubèn Lopez Valés, de la Universidad Autónoma de Barcelona, en cuyo laboratorio se están llevando a cabo los experimentos de daño medular.

A mis compañeros de laboratorio con los que tan buenos momentos he compartido durante estos años. A los que ya estaban cuando llegué y que han contribuido a crear los lazos tan importantes en el funcionamiento de este grupo, Belli, Mar, Ángeles, Rocío, Nono, Moisés, Jorge, Paco, Carlos, Ana, Marta y en especial a Inés, porque de ella he adquirido conocimientos valiosísimos tanto en el laboratorio como para la vida. A los que llegaron después y han sabido mantener el espíritu de compañerismo y de trabajo en equipo, Ana, Sergio, Paloma, Fernando, Nora, y sobre todo a Gloria por sus consejos, y a Javi y Ainoa, que además de excelentes compañeros son grandes amigos que han hecho de esta etapa una época inolvidable. A Cristina y Andrea, cuyos proyectos he tenido el honor de codirigir, por su ayuda y entusiasmo.

A mis padres y amigos, muy especialmente a Alejandro y a Astrid, porque si no fuera por su apoyo, comprensión y cariño no estaría hoy aquí.

TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

Throughout this manuscript, abbreviations and acronyms recommended by the American Chemical Society in the Organic Chemistry and Medicinal Chemistry areas have been employed (revised in the *Journal of Organic Chemistry* and *Journal of Medicinal Chemistry* on January 2017; http://pubs.acs.org/paragonplus/submission/joceah/joceah_authguide.pdf and http://pubs.acs.org/paragonplus/submission/jmcmar/jmcmar_authguide.pdf). In addition, those indicated below have also been used.

ATX	Autotaxin
BSI	Back-scattering interferometry
Caps	Capsaicin
DAPI	4',6-Diamidino-2-phenylindole
DMEM	Dulbecco's modified Eagle medium
DRG	Dorsal root ganglion
EDC	*N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide
Edg	Endothelial differentiation gene
EGFP	Enhanced green fluorescent protein
E_{max}	Maximal receptor activation
FACS	Fluorescence-activated cell sorting
FAF BSA	Fatty acid free bovin serum albumin
FBS	Fetal bovine serum
Glu	Glutamate
HOBt	1-Hydroxybenzotriazole
K_D	Dissociation constant
LP	Lysophospholipid
LPA	Lysophosphatidic acid
LPC	Lysophosphatidylcholine

MAG	Myelin associated glycoprotein
MBP	Myelin basic protein
MPZ	Myelin protein zero
MW	Microwave
N.E.	No effect
NP	Neuropathic pain
PG	Protecting group
PLA	Phospholipase A
PMP22	Peripheral myelin protein 22
PS	Polystyrene supported
RI	Refractive index
ROCK	Rho kinase
S1P	Sphingosine 1-phosphate
s.e.m.	Standard error of the mean
SC	Schwann cell
SCI	Spinal cord injury
SP	Substance P
TBAI	Tetrabutylammonium iodide
TRPV1	Transient receptor potential of type 1 vanilloid channel
VS	Virtual screening

1. INTRODUCTION AND OBJECTIVES

Lysophospholipids (LPs) are cell membrane lipid derivatives that also act as extracellular signals and play important roles in humans and other animals. Analyses of LPs in human body fluids from subjects with different pathophysiological conditions reveal not only the relevance of LPs in human diseases, but also their potential application as biomarkers and/or therapeutic targets. In recent years, the identification and characterization of the receptors for LPs and enzymes regulating their metabolism have facilitated the understanding of their role and signaling properties. Moreover, genetic depletion of LP receptors in mice has provided and will continue to provide critical information on their biological roles. In this manner, specific agonists and antagonists for each LP receptor will be powerful tools not only for basic research, but also for potential therapeutic usages. Among the LPs, lysophosphatidic acid (LPA) stands out as a molecule that elicits a plethora of biological effects, notably within the developing and adult nervous system.[1,2]

Structurally, LPA (1-acyl-*sn*-glycerol-3-phosphate) presents a phosphate head group and a single fatty acyl chain attached to a glycerol backbone. It is found in various body fluids, but it is highly present in plasma, where the most abundant forms are the 16:0, 18:1 and 18:2 (Figure 1).[3] The 18:1 form is the most commonly used as research tool for activation of LPA receptors, and is the one we will refer as LPA along this work.

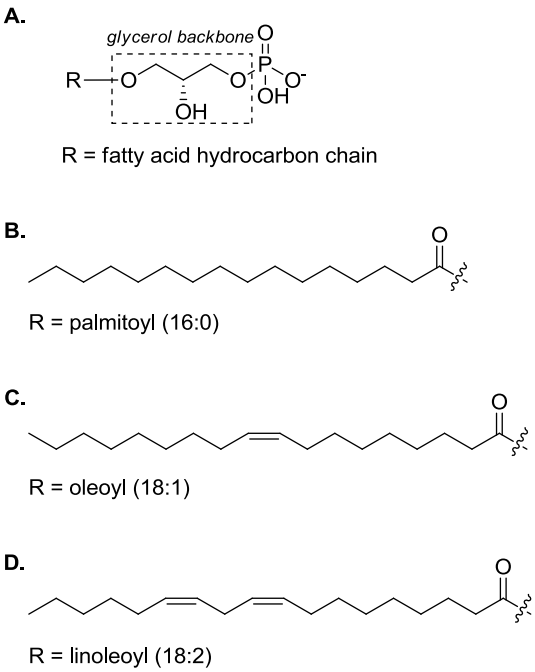


Figure 1. General structure of LPA (A) and different fatty acid hydrocarbon chains: palmitoyl (B), oleoyl (C) and linoleoyl (D).

LPA is produced through several enzymatic pathways. Lysophospholipase D (known as autotaxin, ATX) is the primary enzyme responsible for LPA production in blood, where liberates a choline group from lysophosphatidylcholine (LPC). In addition, phospholipases A1 (PLA_1) and A2 (PLA_2) deacylate phosphatidic acid to produce 2-acyl and 1-acyl LPA, respectively (Figure 2). Importantly, LPA is also *de novo* synthesized from glycerol-3-phosphate by the action of acyltransferases.[4,5]

LPA regulates a wide variety of biological activities, including cellular proliferation, survival and migration, cytoskeletal changes, and cytokine and chemokine mediated cell to cell interaction by binding to specific G protein-coupled receptors (GPCRs).[6,7] Currently there are six LPA ($LPA_{1–6}$) receptors that can be classified into two subgroups: the endothelial differentiation gene (Edg) family and the non-Edg family (Figure 2).[8] The first comprises LPA_1/Edg2,[9,10] LPA_2/Edg4[11] and LPA_3/Edg7[12] receptors that share about 47-51% identities at the amino acid level. By using knockout mice, several important roles have been revealed for Edg family of LPA receptors in the nervous system, cancer, cardiovascular function and reproduction.[13] The non-Edg family includes LPA_4/p2y9/GPR23,[14,15] LPA_5/GPR92/93[16] and LPA_6/p2y5,[17] phylogenetically related to the P2Y purinergic receptor family. They are associated to distinct functions of LPA, including vasculature development, platelet activation and hair growth.[13] In addition, LPA can also bind to the intracellular peroxisome proliferator activated receptor γ (PPARγ)[18] and the transient receptor

potential of the type 1 vanilloid channel (TRPV1),[19] thus playing important roles in gene regulation and nociception, respectively.

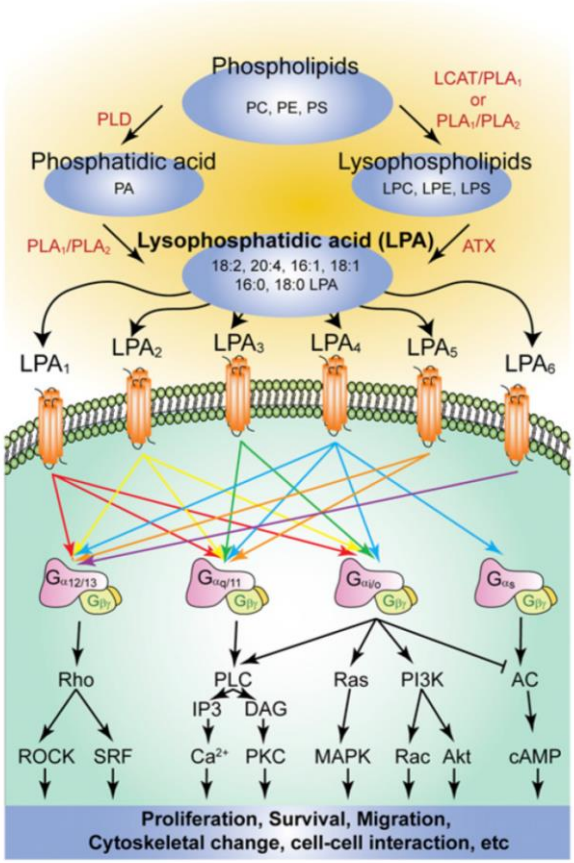


Figure 2. Schematic representation of the major routes of LPA synthesis and the activated signaling pathways via the six cognate LPA receptors. (Source: *J. Lipid Res.* **2014**, *55*, 1192-1214).

To date, a wide range of LPA effects have been identified in the nervous system.[20,21] In fact, in the developing nervous system, LPA plays a critical role during neurogenesis in establishing the cerebral cortex. Furthermore, LPA signaling is involved in neural progenitor cell physiology, astrocyte and microglia activation, neuronal cell death, axonal retraction and development of neuropathic pain.[22] Considering the pleiotropic effects of LPA on many key nervous system cell types, together with data showing localized up-regulation of LPA receptors after injury in mice and humans, it is likely that LPA regulates essential aspects of the cellular reorganization after neural trauma.

Among all neuropathologies where LPA plays an important role, neuropathic pain (NP) and spinal cord injury (SCI) are high incidence and seriously disabling conditions which currently lack specific pharmacological therapies. LPA_1 and LPA_2 receptor

subtypes have been functionally linked to many neural processes associated to these pathologies, but the lack of potent and selective agonists and antagonists has impaired the delineation of their specific role. Hence, we have focused our efforts on the development of such agents to clarify the biological role of LPA_1 and LPA_2 receptors in NP and SCI.

1.1. Neuropathic Pain

NP is a form of chronic pain affecting millions of people worldwide and it is associated with very significant loss in terms of life quality, including the ability to work and to work efficiently. It is not caused through stimulation of sensory neurons by "physiological" pain stimuli but rather by dysfunction or injury of the fibers. This dysfunction commonly occurs as a secondary symptom in diseases such as diabetes, cancer, and multiple sclerosis or as a side effect of chemotherapeutic treatments. Characteristic symptoms include unpleasant abnormal sensations (dysaesthesia), an enhanced perception of pain in response to noxious stimuli (hyperalgesia), as well as abnormal painful responses to innocuous or tactile stimuli that do not usually cause pain (allodynia). There are multiple molecular mechanisms underlying the pathophysiology of NP, thus increasing the complexity of developing effective targeted therapeutics. Currently approved treatments are focused on the inhibition of pain trasmission.[23] The medication includes the antidepressants duloxetine and desipramine,[24] as well as the anticonvulsivants gabapentin and pregabalin.[25] The first two drugs produce their analgesic effects by preventing presynaptic reuptake of serotonin and noradrenaline, both of which inhibit the descending pain pathways in the central nervous system (CNS). On the other hand, gabapentin and pregabalin decrease the release of the excitatory neurotransmitters noradrenaline, glutamate (Glu), and substance P (SP). However, current therapeutics usually need to be taken chronically and many patients do not achieve a satisfactory response or observe intolerable side effects.[26]

Among many processes that have been described to play a role in NP, an extensive body of work points to a role for LPA signaling.[27-29] In the last decade, it was demonstrated that, after peripheral nerve damage, LPA produced in post-synaptic neurons is able to activate macrophage cell types through LPA_3 receptor, initiating *de novo* production of LPA via the enzymatic activity of c/iPLA_2 and ATX (Figure 3).[30-34] In this manner, the high concentration of LPA is responsible for demyelination and central sensitization, through LPA_1 receptor, and TRPV1 activation, considered the three key mechanisms for the initiation and maintenance of NP.[35-40]

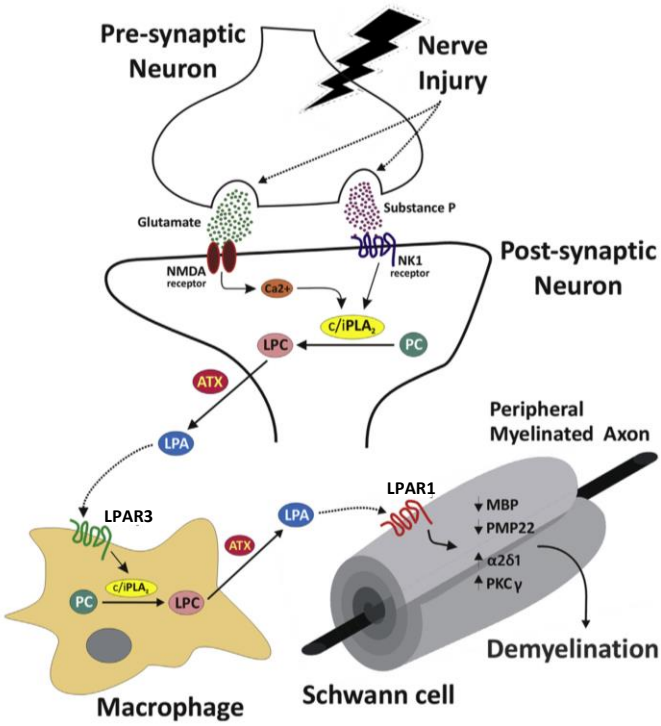


Figure 3. Schematic representation of LPA production via LPA_3 receptor activation following intense stimulation of primary afferent neurons. (Source: *Neuropharmacology* **2016**, *113*, 608-617).

a) Demyelination

Myelinating Schwann cells (SC) form the myelin sheath around axons of motor and sensory neurons. They are essential for the proper functioning of nervous system, such as the conduction of nervous impulses along axons, nerve development and regeneration. During NP, the high level of LPA provokes a damage to the myelin sheath via LPA_1 receptor, which occurs through two pathways; a rapid-acting non genomic pathway, that involves calpain-mediated myelin associated glycoprotein (MAG) degradation,[41] and a late phase that involves transcriptional repression of myelin-related genes.[42-46] The loss of MAG results in disinhibition of sprouting, while the downregulation of compact myelin proteins leads to a loosening of the myelin sheath (Figure 4). It seems that the demyelination may underlie the mechanisms for allodynia.

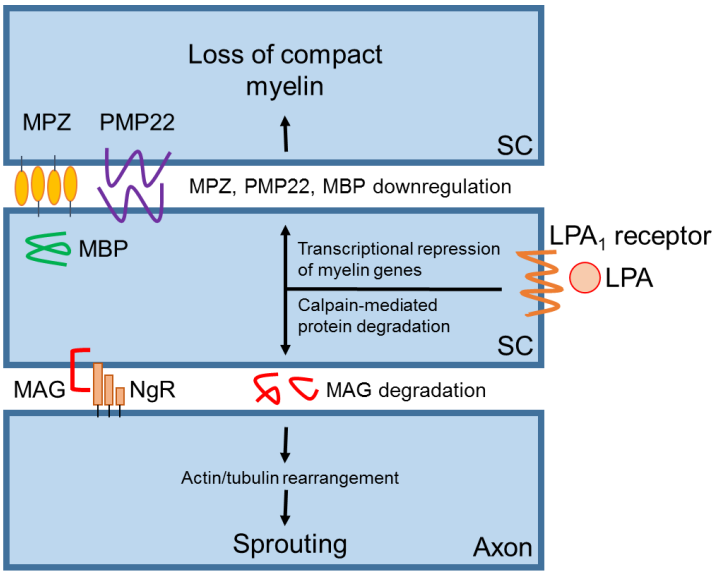


Figure 4. Schematic representation of LPA_1 receptor-mediated demyelination and sprouting. Myelin basic protein (MBP), myelin protein zero (MPZ) and peripheral myelin protein 22 (PMP22) have a role in the architecture of compact myelin, while MAG is restricted to the innermost membrane facing the neuronal axon through NgR complex.

b) Central sensitization

The dorsal root ganglion (DRG) contains the cell bodies of sensory neurons that help transmit the sensory messages of pain and touch from the periphery to the spinal cord. During NP, the high level of LPA alters this impulse transmission via LPA_1, leading to hyperalgesia (Figure 5).[47,48]

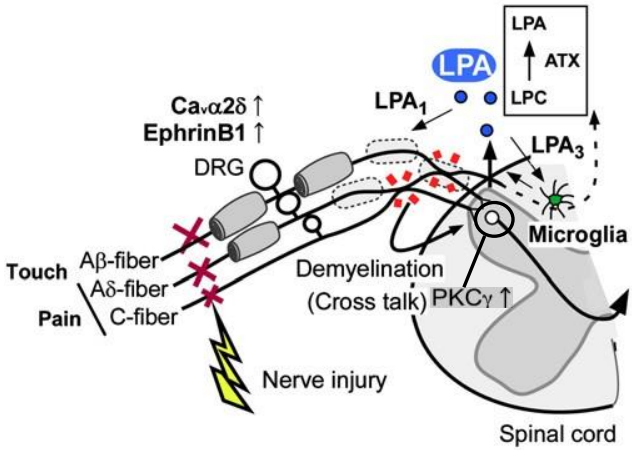


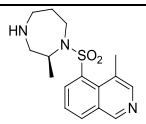
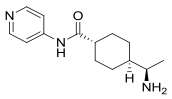
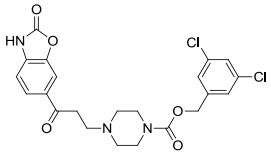
Figure 5. Schematic representation of $LPAR_1$-mediated upregulation of $Ca\alpha_2\delta_1$ and $EphrinB_1$ in DRG and PKCγ in dorsal horn, leading to hyperalgesia. (Source: *Biochim Biophys Acta* **2013**, *1831*, 61-73).

c) Involvement of TRPV1 in NP

TRPV1 is activated by a variety of stimuli such as high temperature, low pH and pungent compounds. Also, multiple signals that originate from inflammatory processes converge on TRPV1, whose activation in sensory neurons has the final consequence of pain perception.[49] In 2000, it was reported that TRPV1 knockout mice showed a significant blockade of the thermal hyperalgesia,[50] and recently, it was demonstrated direct interaction of TRPV1 with LPA,[19] suggesting a role for this channel in the development of NP, but the mechanisms of actions are still to be clarified.

In summary, several signaling pathways are responsible of the NP development. Although many efforts have been made in order to interfere in these pathways, none of the synthesized compounds has progressed to a marketed drug, but they remain as valuable research agents. Among them, the most important are the Rho kinase (ROCK) inhibitors, H-1152[51] and Y-27632,[52] involved in the downstream of LPA signaling (see Figure 2), and the ATX inhibitor, PF-8380 (Table 1).[53]

Table 1. Research agents for the study of NP.

Name	Structure	Mechanism of action	Drawback
H-1152		ROCK inhibitor	Excessive doses are required for sustained analgesic effect.[51]
Y-27632		ROCK inhibitor	Lack of ROCK selectivity.[54]
PF-8380		ATX inhibitor	Lack of improvement of the anti-inflammatory potential with respect to naproxen.[53]

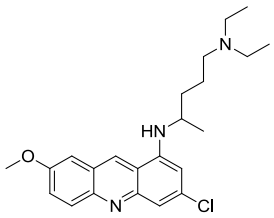
Given the implication of LPA_1 receptor in NP, a strategy could be targeting this receptor with antagonists to block the signaling pathway. However, the efficacy of antagonists is usually limited, since they are only effective for the first 2-4 hours after administration. Hence, one alternative could be targeting LPA_1 receptor with a selective agonist, which should promote receptor internalization and desensitization, and accordingly, could produce long-lasting antinociceptive effects. Although several structure-activity relationship (SAR) studies had been carried out, no potent ligands for the LPA_1 receptor had been described, especially in the agonist field. Towards this end, in this work we will address the development of potent and selective LPA_1 receptor agonists, as antinociceptive agents.

1.2. Spinal Cord Injury

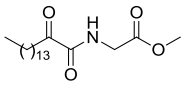
Millions of people worldwide suffer from SCI, which is considered as one of the most physically disabling and psychologically devastating conditions known to humans. Moreover, the majority of victims are young and otherwise healthy individuals.

Functionally, the spinal cord is the part of the CNS that controls the transmission of neural signals between the brain and the rest of the body. Although the spinal cord is well protected by the bone of the spinal column, it can be damaged by mechanical trauma or, less frequently, injured by infection, insufficient blood flow, or diseases that alter its nerve function. Any of these spinal cord injuries result in a disruption of the pathways that carry information between brain and body, leading to possible serious disability, as well as of neuronal networks related to many physiological functions, including the respiratory, gastrointestinal, urinary, and autonomic nervous system. The pathophysiology of SCI is widely described as biphasic. The primary phase involves the initial mechanical trauma and the subsequent neurological deficits, while the secondary phase results in a prolonged period of tissue destruction, contributing to permanent functional disabilities.[55] Primary injury cannot be avoided, therefore comprehension of secondary injury mechanisms in SCI are invaluable requisite for planned therapeutic strategies, in order to improve neural regeneration and promote functional recovery. To date, only limited effective treatments are available.

Pharmacological inhibition of Rho/ROCK pathway and PLA_2 enzyme were studied in order to prevent cell death and promote axon regeneration after SCI. Many efforts have been made in this sense, but only a compound seems to be a promising drug; BA-210 (trademarked as Cethrin®) blocks activation of Rho and is in phase-II/III clinical trials to treat SCI.[56]



BA-210



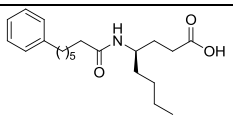
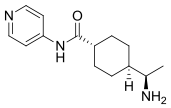
AX-115

Also, the pan-PLA_2 inhibitor, AX-115[57-60] is likely to be excellent candidate for drug development for the treatment of SCI, while the $sPLA_2$ inhibitor, GK115,[58-60] and the ROCK inhibitor, Y-27632,[61] are used mainly as research agents for the study of SCI (Table 2).

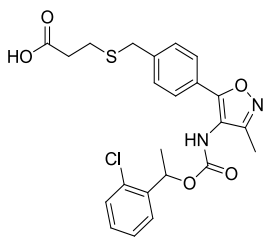
The inflammatory response that occurs after SCI strongly contributes to secondary injury. In this context, LPA is considered as an important mediator of inflammation.[62] In fact, a recent study suggests that the increased level of LPA in the

injured spinal cord is implicated in demyelination and loss of function by signaling via LPA_1 and LPA_2 receptors, contributing to secondary damage.[63-65] Moreover, two recent works confirmed this evidence, showing a reduced tissue damage after spinal cord hemisection injury in mice[66] and brain trauma[67] using the anti-LPA monoclonal antibody, LT3114, that blocks LPA interaction with its receptors. This antibody is currently under investigation as therapeutic agent, given that it recognizes all relevant LPA species, including the unusual 18:0 and 16:0 LPAs that are abundant in the cerebrospinal fluid of injured mice and humans (Table 2).[66,67]

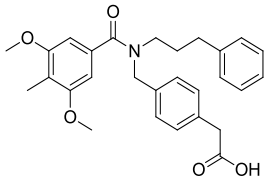
Table 2. Research agents for the study of SCI.

Name	Structure	Mechanism of action	Drawback
GK115		$sPLA_2$ inhibitor	Selective only for $sPLA_2$.[58]
Y-27632		ROCK inhibitor	Inhibition ability is not selective for ROCK, but for different protein kinases.[54]
LT3114	Antibody	LPA antibody	Competition with LPA receptors for LPA.[67]

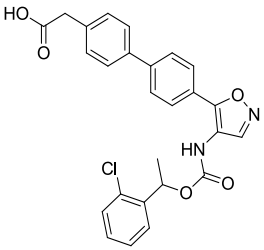
Hence, the contribution of LPA signaling in the secondary damage after SCI is recognized and, among the LPA receptors, LPA_1 and LPA_2 seem to be implicated.[68] Currently, there are a number of LPA_1 receptor antagonists in early pre-clinical phase studies for the treatment of cancer (Ki16425[69] and ONO-7300243[70]), fibrosis (AM966[71] and AM095,[72] the *R* stereoisomer of AM966) and systemic sclerosis (SAR100842,[73] structure undisclosed). However, only two compounds are specifically indicated for treating CNS injury; BMS-986202, in phase I, and BMS-986020, in phase II clinical trials, whose structures have not been disclosed yet.[74]



Ki16425



ONO-7300243



AM966

Given that little is known about the specific involvement of LPA_1 and, especially, LPA_2 receptors in the pathophysiology of SCI, there is a need to elucidate the role of these receptors. Towards this end, in this work we have considered the complementary action of LPA_1 and LPA_2 receptors, focusing our investigation on targeting both receptors with antagonists.

In summary, the objective of this work is the design and synthesis of new potent and selective LPA_1 agonists that allow the validation of this approach to ameliorate NP, and the development of new LPA_1 and LPA_2 selective antagonists to study the role of these receptors in decreasing the damage associated to SCI. This overall objective involves the following steps:

1. Design and synthesis of new ligands with agonist or antagonist activity at LPA_1 and LPA_2 receptors.
2. Determination of the activity and selectivity at LPA receptors.
3. Biological validation of the selected compounds.

RESULTS AND DISCUSSION

2. RESULTS AND DISCUSSION

2.1. Design and synthesis of new agonists for the LPA_1 receptor

Binding of an agonist to a GPCR leads to the activation of heterotrimeric G proteins, which in turn stimulates or inhibits effector proteins. In the case of prolonged or repeated stimulation, GPCRs are phosphorylated by GPCR kinases and recruit β-arrestins, events responsible for fast signal desensitization. Subsequently, GPCRs are often internalized into endosomes, where they are either degraded or dephosphorylated and recycled back to the cell surface to sustain a new cycle of activation (Figure 6). This regulatory mechanism allows for the interruption of prolonged GPCR activation and the excessive amplification of the respective signaling pathways engaged in the response.[75-77] The desensitization process can be the result of the own downregulation of the activated receptor (homologous desensitization), or it can be related to modulation of a pathway common to other receptors (heterologous desensitization). Currently it is known that many GPCRs can be phosphorylated, desensitized, and internalized by both agonist stimulation and in a heterologous manner. Generally, GPCR desensitization can limit the therapeutic usefulness of many receptor agonists, but there are cases where this reduced sensitivity is the aim of the research. One example is the sphingosine 1-phosphate (S1P) receptor modulator FTY720, marketed as Fingolimod® for the treatment of multiple sclerosis.[78] It binds with high affinity to S1P receptor and mediates its cellular effects through agonist activation, followed by internalization of the receptors as a result of its potent stimulation.

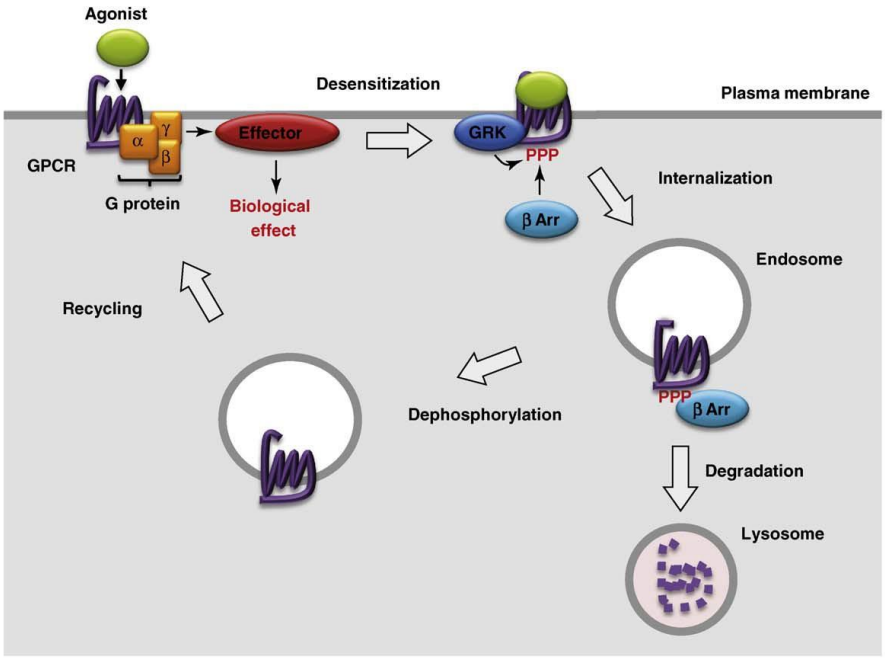


Figure 6. Representation of GPCR desensitization and internalization processes (Source: *Trends Pharmacol. Sci.* **2010**, *31*, 221-228).

In 2003, a study using human LPA_1 receptor-transfected HeLa cells showed that treatment with LPA induced rapid endocytosis of approximately 40% of surface LPA_1 receptors within fifteen minutes.[79] After that, several groups have elucidated the desensitization and internalization mechanisms of LPA_1 receptor after stimulation with an agonist.[80,81] Hence, it is possible that activation of LPA_1 receptor with an agonist could lead to the desensitization of the receptor and accordingly block the signal transduction, which could translate into a blockade of neuropathic pain, one of the objective of this work. Therefore, in this context, we focused our efforts on finding new molecular entities with agonist activity at LPA_1 and able to induce receptor desensitization.

At the moment of starting this project, the 3D structure of the LPA_1 receptor had not been elucidated and no potent and selective ligands for the LPA_1 receptor had been described, especially in the agonist field. Hence, the structure of the endogenous ligand, LPA, was used as starting point. Initially, two series of compounds were designed: series **I**, which comprised changes in the acid group attached to the glycerol backbone of LPA, and series **II**, which included modifications in the hydrophobic chain (Figure 7).

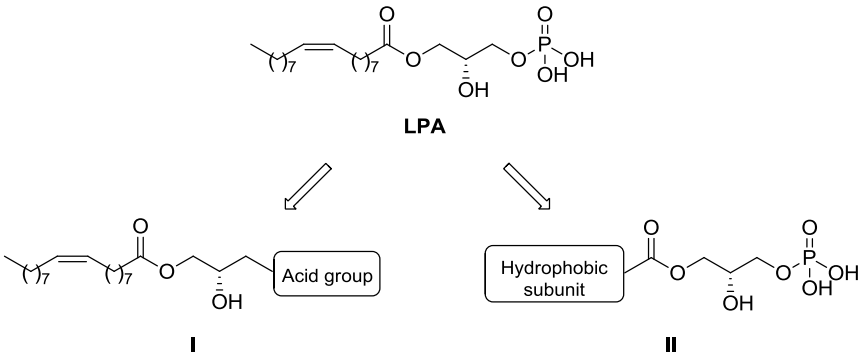


Figure 7. Design of new LPA_1 ligands.

2.1.1. Design and synthesis of series **I**

Several SAR studies[82] suggest that changes in the polar head of LPA are poorly tolerated. In particular, it has been shown that an important requirement for activity is the presence of free acid groups retaining their negative charge under physiological conditions.[83] In fact, when the phosphate group has been modified in LPA-like structures, it has usually led to a decrease in LPA_1 activity of agonists. This is confirmed by the few potent agonists described for this receptor despite the variety of phosphate modifications tried.

Accordingly, phosphorous-free isosters, specifically carboxylic and boronic acids, and tetrazole (Figure 8) were chosen as acidic subunits in compounds of series **I**, as they have been described as phosphoryl replacements.[84,85] The synthesis of the designed compounds implied the preparation of the corresponding glycerol derivatives for their subsequent regioselective coupling with the oleate moiety (Figure 8). It must be noted that in all cases the stereochemistry was conserved identical to LPA.

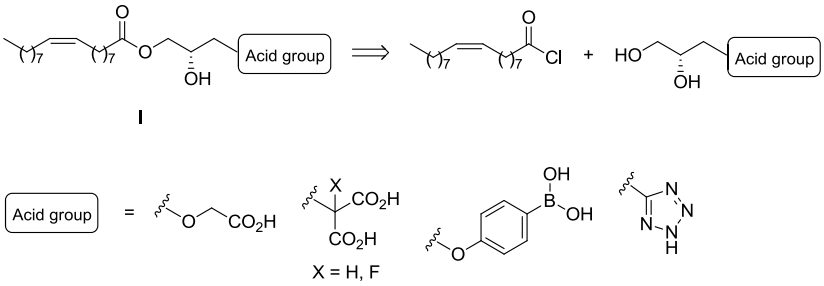


Figure 8. Acid groups proposed for series **I**.

The synthesis of the glycerol derivatives functionalized with a carboxylic acid started by choosing the adequate and compatible protecting groups for the diol and the carboxylic acid moieties present in these compounds (Figure 9). The two groups had to be orthogonal, this is, it was required that the deprotection of the diol did not

affect the protecting group of the carboxylic acid. Additionally, ultimate deprotection of the carboxylic acid had to be compatible with the presence of the oleate ester group in the final compounds. Therefore, *tert*-butyl (*t*Bu) ester was chosen as protecting group of the carboxylic acid, due to its easy cleavage in acid media which should not affect the base-labile oleate ester. Regarding the glycerol, the benzyl group (Bn) was chosen as protecting group of the diol since its removal by hydrogenation would not affect the *t*Bu ester.

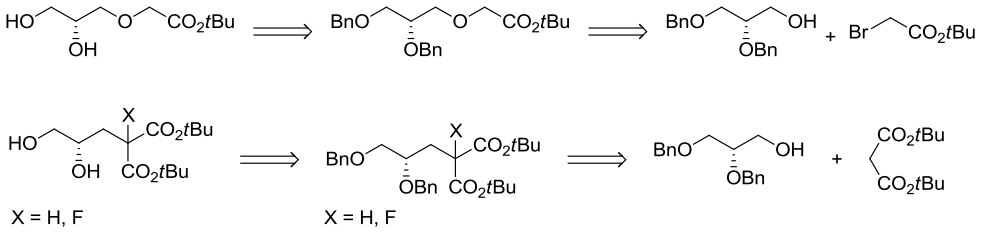


Figure 9. Retrosynthetic analysis for the carboxylic acid glycerol derivatives.

In the case of the glycerol derivative bearing a boronic acid, the need of two orthogonal protecting groups was solved by protecting the diol as an isopropylidene acetal, whereas the boronic acid was introduced as a pinacol ester, whose deprotection conditions should be compatible with the presence of the oleate moiety (Figure 10).

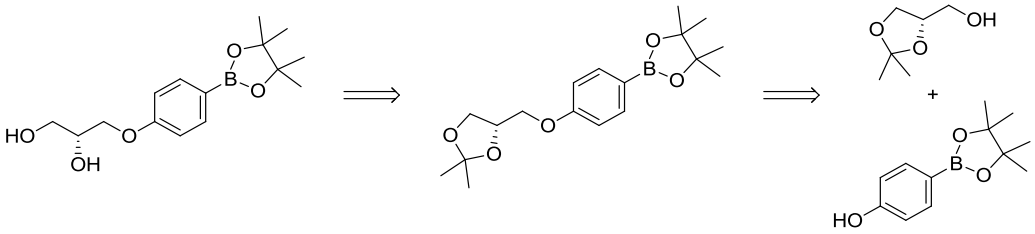


Figure 10. Retrosynthetic analysis for the boronic acid glycerol derivative.

Regarding the tetrazole derivative, the diol was also protected as an acetal, and the primary hydroxyl group was transformed into a cyano group for its later conversion to the corresponding tetrazole (Figure 11).

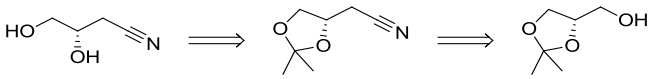
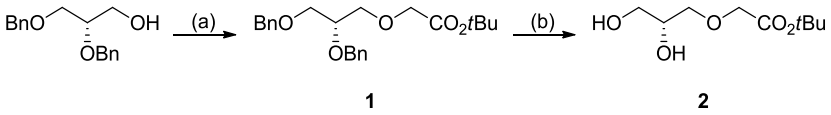


Figure 11. Retrosynthetic analysis for the cyano glycerol derivative.

Starting with the carboxylic acid derivatives, alkylation of commercially available (*S*)-(-)-2,3-bis(benzyloxy)propan-1-ol with *tert*-butyl bromoacetate in the presence of sodium hydride (NaH) and tetrabutylammonium iodide (TBAI) led to compound **1**,

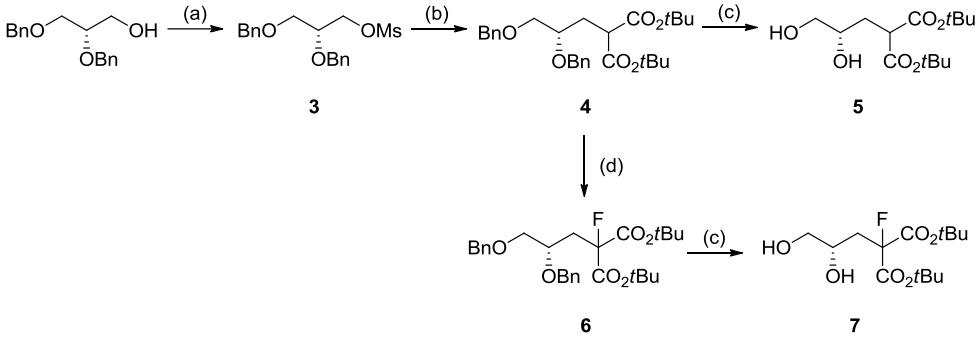
which was deprotected by catalytic hydrogenation to afford the glycerol intermediate **2** (Scheme 1).



1 **2**

Scheme 1. Reagents and conditions: (a) *tert*-Butyl bromoacetate, NaH, TBAI, THF, 0ºC to 50ºC, 16 h, 22%; (b) H_2, 10% Pd(C), EtOH, 60ºC, 95%.

Next, glycerol derivatives functionalized with malonic and α-fluoromalonic acid were prepared, where the fluorine atom was introduced in order to increase the acidity of the carboxylic acids. Thus, (*S*)-(-)-2,3-bis(benzyloxy)propan-1-ol was transformed into the corresponding mesylate **3**, which was then alkylated with di-*tert*-butyl malonate using NaH as base to yield intermediate **4**. For the preparation of the α-fluoromalonic derivative, intermediate **4** was treated with Selectfluor® under basic conditions. Catalytic hydrogenation of intermediates **4** and **6** afforded the glycerol derivatives **5** and **7** (Scheme 2).

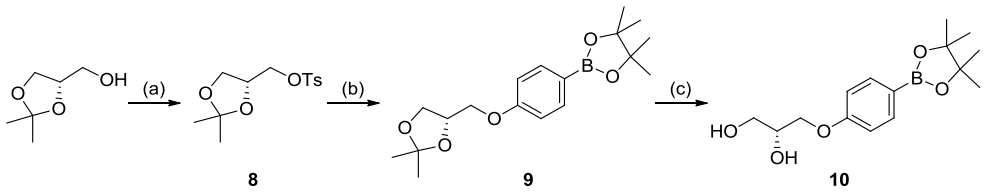


3 **4** **5**

6 **7**

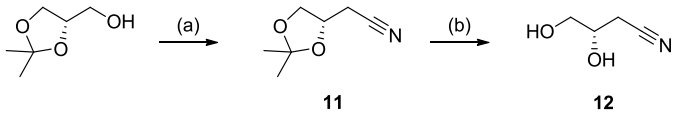
Scheme 2. Reagents and conditions: (a) Mesyl chloride, Et_3N, DCM, 0ºC to rt, 1 h, 80%; (b) di-*tert*-butyl malonate, NaH, NaI, DMF:THF, 0ºC to 80ºC, 17 h, 76%; (c) H_2, 10% Pd(C), EtOH, 60ºC, 89-95%; (d) Selectfluor®, NaH, DMF:THF, 0ºC to rt, 48 h, 99%.

For the glycerol derivative bearing a boronic acid moiety (**10**), the synthesis started with the tosylation of commercially available (*S*)-(+)-1,2-isopropylydeneglycerol [(*S*)-solketal] to obtain intermediate **8**, which was further reacted with 4-hydroxyphenylboronic acid pinacol ester to yield derivative **9**. Diol **10** was obtained by deprotection of the acetal using polystyrene-supported *p*-toluensulfonic acid (PS-*p*TsOH) (Scheme 3).



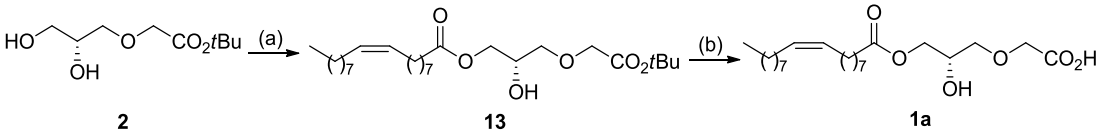
Scheme 3. Reagents and conditions: (a) Tosyl chloride, pyridine, DCM, 0ºC to rt, 16 h, 86%; (b) 4-hydroxyphenylboronic acid pinacol ester, Cs_2CO_3, DMF, 90ºC, 16 h, 84%; (c) PS-*p*TsOH, CH_3OH, rt, 18 h, 88%.

The glycerol intermediate funcionalized with a cyano group was synthesized by transformation of (*S*)-solketal into the corresponding triflate, followed by treatment with potassium cyanide to afford nitrile **11**. Diol **12** was obtained by cleavage of the acetal using trifluoroacetic acid (TFA) (Scheme 4).



Scheme 4. Reagents and conditions: (a) i) Pyridine, triflic anhydride, DCM, -20ºC, 30 min; ii) KCN, CH_3CN:H_2O, rt, 12 h, 99%; (b) TFA, CH_3OH, rt, 1.5 h, 61%.

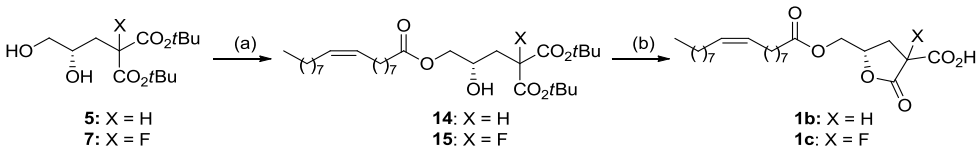
Once the functionalized glycerol derivatives **2**, **5**, **7**, **10**, and **12** were synthesized, their regioselective condensation with oleic acid was carried out. Following reaction conditions previously employed by our group, **2** was coupled with oleoyl chloride in the presence of 2,4,6-collidine at low temperature to afford ester **13**. The reaction was completely regioselective, and only the acylation at *sn*-1 position of the glycerol moiety was observed. The *tert*-butyl group of intermediate **13** was removed by treatment with TFA, yielding the final compound **1a** without detection of the hydrolysis of the oleate ester (Scheme 5).



Scheme 5. Reagents and conditions: (a) Oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 36%; (b) TFA, DCM, rt, 17 h, 52%.

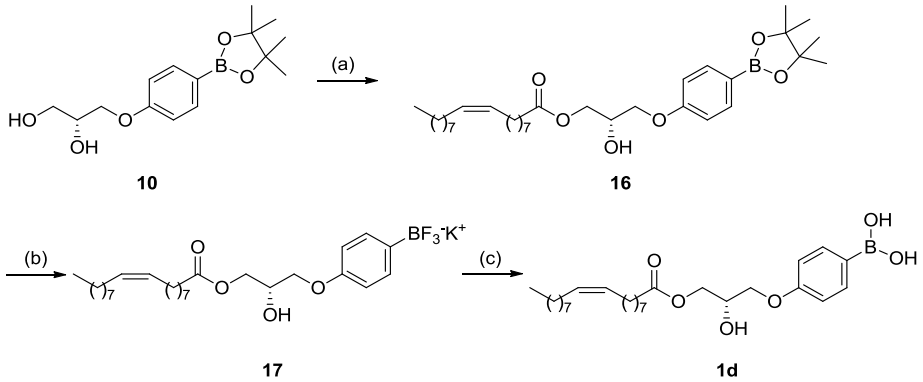
The esterification conditions were then applied to the reaction of the oleoyl chloride with the malonate derivatives **5** and **7** to yield the intermediate esters **14** and **15** which were then subjected to acidic conditions to remove the *tert*-butyl group. In this case, the treatment of derivatives **14** and **15** with TFA led to the formation of lactones **1b** and **1c** instead of the expected malonic acids (Scheme 6). These

products, obtained as a 1:1 mixture of diastereoisomers, are the consequence of the intramolecular cyclization of the carboxylic acid with the hydroxy group in γ position.



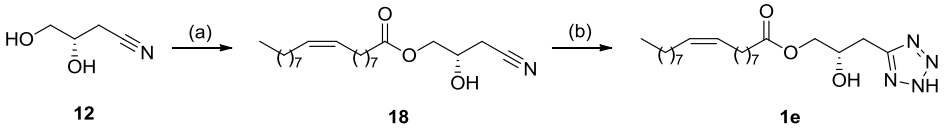
Scheme 6. Reagents and conditions: (a) Oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 60-99%; (b) TFA, DCM, rt, 17-18 h, 88-99%.

The esterification conditions were also used to carry out the reaction of glycerol derivative **10** with oleoyl chloride. The pinacolyl boronate ester **16** was transformed into the corresponding trifluoroborate salt **17**, followed by hydrolysis with trimethylsilyl chloride (TMSCl) to yield final compound **1d** (Scheme 7).



Scheme 7. Reagents and conditions: (a) Oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 40%; (b) KHF_2, CH_3OH:H_2O, rt, 30 min, 99%; (c) TMSCl, CH_3CN:H_2O, rt, 1 h, 60%.

Finally, diol **12** was coupled with oleoyl chloride to obtain ester **18**, which led to tetrazole derivative **1e** by cycloaddition reaction with sodium azide, under microwave (MW) irradiation (Scheme 8).



Scheme 8. Reagents and conditions: (a) Oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 12 h, 88%; (b) NaN_3, NH_4Cl, DMF, MW, 160ºC, 45 min, 5%.

Once the compounds **1a**-**e** were synthesized, they were tested in a LPA_1-overexpressing cell line, to determine if any of the phosphate replacements carried out had allowed to obtain compounds with activity at this receptor.

2.1.2. Determination of the agonist activity at LPA_1 receptor

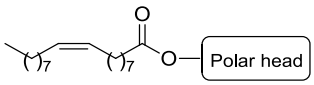
In order to test the synthesized compounds as LPA_1 receptor agonists, the corresponding biological assay was set up in our laboratory. The ability of the compounds to activate the receptor was determined by the measurement of calcium mobilization in RH7777 cells stably transfected with the receptor, as the binding of an LPA_1 agonist causes an increase in intracellular calcium levels,[86] which can be quantified using a fluorescence-based assay.

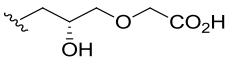
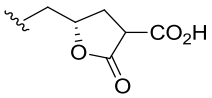
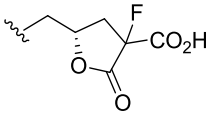
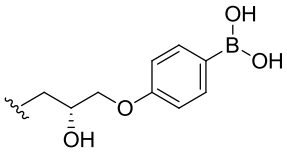
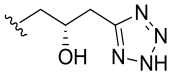
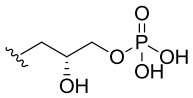
After some experimental optimizations, Fluo 4-NW (no-wash) was chosen as the most appropriate calcium sensor. To determine the agonist activity of the compounds, cells were preloaded with Fluo 4-NW and then incubated in the presence of the compounds under study. Fluorescence intensity was measured (excitation wavelength of 494 nm and emission wavelength of 516 nm) in a 96-well microplate reader. Ionomycin (10 µM) and LPA were employed as positive controls, with 10 µM LPA response being 30% of ionomycin response.[87] In order to rule out non-LPA_1 receptor mediated calcium mobilization, parallel experiments using non-transfected RH7777 cells were carried out.

The compounds were initially tested at a fixed dose of 10 µM and the maximal receptor activation (E_{max}) for each of them was expressed as a percentage, referring their response to that induced by 10 µM LPA. In order to obtain the half maximal effective concentration (EC_{50}) value for those compounds that presented E_{max} over 30%, the complete dose-response curve, at six or seven different concentrations of the ligand, was determined. Data are expressed as the average and standard error (s.e.m.) obtained from two to four independent experiments carried out in triplicate.

The obtained results for compounds **1a**-**e** are shown in Table 3.

Table 3. Agonist activities of compounds **1a-e** at LPA_1 receptor



Compound	Polar head	E_{max} (%)[a]	EC_{50} (μM)[b]	pK_a[c]
1a		N.E.[d]	-	3.4 ± 0.1
1b		N.E.	-	2.7 ± 0.4
1c		33 ± 5	1.7 ± 0.2	0.5 ± 0.4
1d		N.E.	-	8.7 ± 0.2
1e		N.E.	-	5.0 ± 0.1
LPA		100	0.83 ± 0.02	1.8 ± 0.1

[a]E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [b]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [c]Values estimated with ACDLabs program. [d]No effect was observed at the highest concentration of compound tested (10 μM).

In this initial set of compounds, the influence of pK_a seems to be important for activity, as only compound **1c**, with a pK_a value lower than LPA, activates the receptor (Table 3). Accordingly, it was necessary to confirm if the non-cyclic α-fluoromalonic derivative originally proposed, with two free carboxylic acids, would exhibit better activity at LPA_1 receptor. In order to avoid the intramolecular cyclization, the most straightforward structural modifications were either the removal of the hydroxy group (compound **1f**) or its masking with a methyl group, as in derivative **1g** (Figure 12).

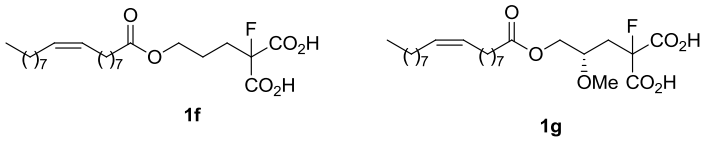
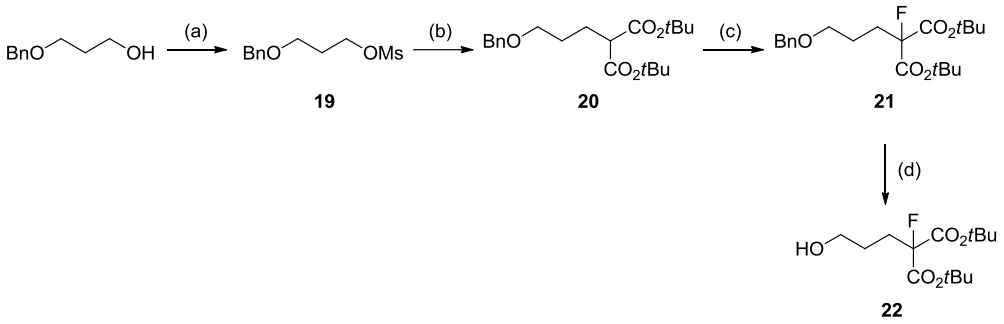


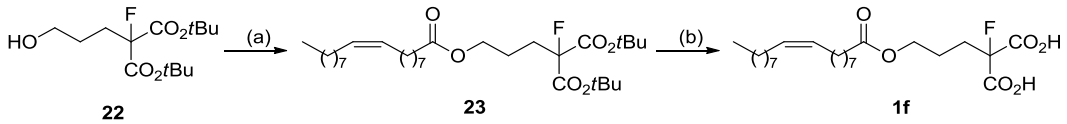
Figure 12. α-Fluoromalonic derivatives.

Hence, the commercially available 3-(benzyloxy)propan-1-ol was transformed into the corresponding mesylate **19**, which was further alkylated with di-*tert*-butyl malonate to obtain intermediate **20**. Treatment of this compound with Selectfluor® to yield fluorinated derivative **21**, and subsequent catalytic hydrogenation to remove the benzyl group afforded the desired alcohol **22** (Scheme 9).



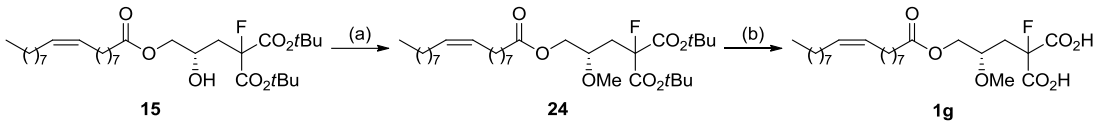
Scheme 9. Reagents and conditions: (a) Mesyl chloride, Et_3N, DCM, 0ºC to rt, 1 h, 99%; (b) di-*tert*-butyl malonate, NaH, NaI, DMF:THF, 0ºC to 80ºC, 17 h, 66%; (c) Selectfluor®, NaH, DMF:THF, 0ºC to rt, 48 h, 99%; (d) H_2, 10% Pd(C), EtOH, 60ºC, 95%.

Once malonate intermediate **22** was synthesized, acylation with oleoyl chloride followed by hydrolysis with TFA allowed to obtain final compound **1f** in good yield (Scheme 10).



Scheme 10. Reagents and conditions: (a) Oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 70%; (b) TFA, DCM, rt, 16 h, 90%.

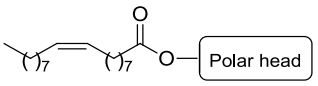
Synthesis of final compound **1g** started from derivative **15**, which by reaction with trimethylsilyldiazomethane and subsequent hydrolysis of the *tert*-butyl ester groups led to the desired malonic derivative (Scheme 11).

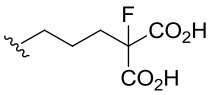
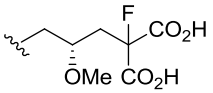
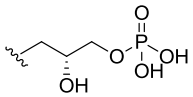


Scheme 11. Reagents and conditions: (a) Trimethylsilyldiazomethane, HBF_4, DCM, 0ºC, 90 min, 38%; (b) TFA, DCM, rt, 17 h, 95%.

The capacity of compounds **1f**,**g** to activate LPA_1 receptor was determined and the results are shown in Table 4. Taking together all the data obtained for series **I**, the necessity of an acidic polar group seems clear. However, it is not the only requirement, as **1f**, with a pK_a value similar to compound **1g**, is completely inactive. This suggests the importance of the oxygen atom at the *sn*-2 position of the spacer that separates the fatty acid chain and the polar head group.

Table 4. Agonist activities of compounds **1f,g** at LPA_1 receptor



Compound	Polar head	E_{max} (%)[a]	EC_{50} (μM)[b]	pK_a[c]
1f		N.E.[d]	-	1.1 ± 0.4
1g		74 ± 14	6 ± 1	0.9 ± 0.4
LPA		100	0.83 ± 0.02	1.8 ± 0.1

[a]E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [b]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [c]Values estimated with ACDLabs program. [d]No effect was observed at the highest concentration of compound tested (10 μM).

In conclusion, this initial series of compounds allowed to identify two LPA_1 receptor agonists, **1c** (E_{max} = 33%; EC_{50} = 1.7 μM) and **1g** (E_{max} = 74%; EC_{50} = 6 μM), which confirms that it is possible to mimic the phosphate group of LPA with other polar moieties. However, these two agonists are still less potent than LPA so, in order to optimize the structure, the study of the influence of the hydrophobic chain of LPA was addressed.

2.1.3. Design and synthesis of series **II**

During the last years, several studies have been carried out trying to establish the structural requirements needed for the hydrophobic moiety. Although no definitive conclusions have been reached yet, it seems clear that modifications on this part of the molecule are less critical for activity at LPA_1 receptor, probably because its flexible disposition facilitates its fitting into the receptor pocket.

Thus, for series **II**, a comprehensive study of the influence of the hydrophobic moiety was carried out, including modifications on the overall length of the fatty acid chain as well as the incorporation of aromatic rings (Figure 13). The designed compounds were prepared by regioselective acylation of the properly protected (PG) phosphorylated glycerol backbone with the appropriate hydrophobic subunit.

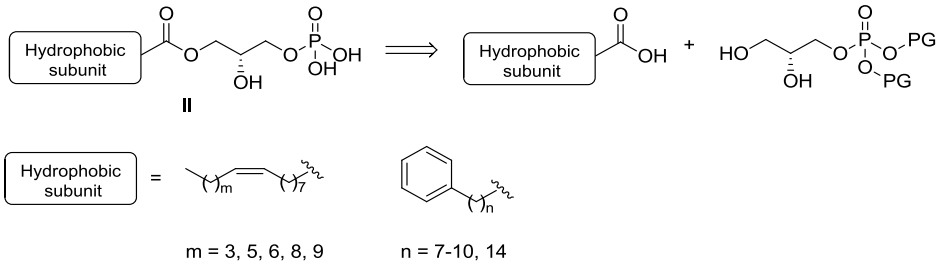


Figure 13. Design of compounds of series **II**.

2.1.3.1. Derivatives of series **II** containing aliphatic chains

Modifications of the length of the fatty acid chain implied the synthesis of the corresponding alkynyl derivatives, followed by their partial hydrogenation to obtain the *Z*-alkenes. Regarding the protecting groups of the phosphorylated glycerol fragment, the compatibility between the deprotection conditions of the phosphate group and the existence of double bonds in the molecule had to be considered, and so, *t*Bu group was chosen, as its hydrolysis takes place in acid media, which should not affect the double bond. Furthermore, the deprotection conditions of the diol should be orthogonal with the *t*Bu phosphate ester, and benzyl group was selected as the most appropriate option (Figure 14).

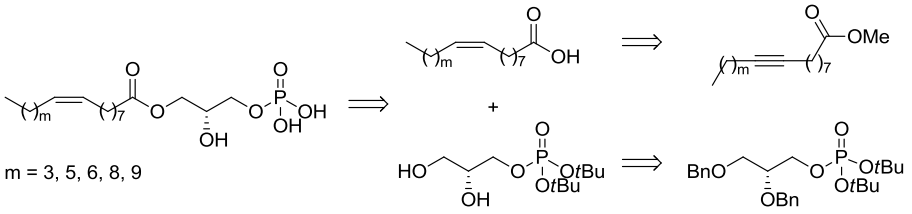
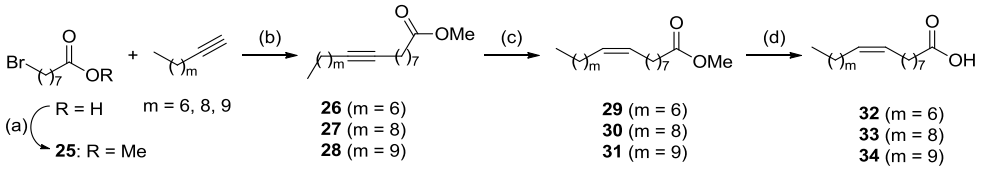


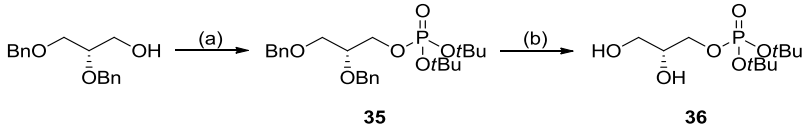
Figure 14. Retrosynthetic analysis for aliphatic derivatives of series **II**.

Alkynyl derivatives **26**-**28** were achieved by a Sonogashira reaction of methyl 8-bromooctanoate (**25**) and the corresponding commercially available alkyne using a carbene-based palladium catalyst suitable for cross-coupling of unactivated alkyl bromides (Scheme 12).[88] The subsequent stereoselective *cis*-hydrogenation of the alkynes **26**-**28** was carried out by Brown reaction[89] under conditions previously optimized in the laboratory. Thus, the desired alkenes **29**-**31** were obtained with total conversion employing nickel acetate and sodium borohydride as the catalytic system, in the presence of ethylendiamine and under a hydrogen atmosphere. Then, the alkenes were hydrolyzed to their corresponding carboxylic acids **32**-**34** (Scheme 12).



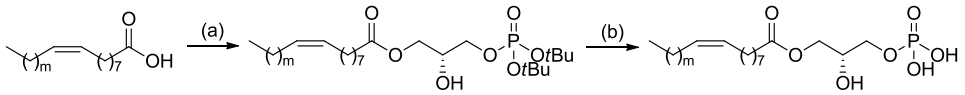
Scheme 12. Reagents and conditions: (a) *p*TsOH, CH_3OH, reflux, 18 h, 95%; (b) $[(\pi\text{-allyl})PdCl]_2$, 1,3-bis(1-adamantyl)imidazolium chloride, CuI, Cs_2CO_3, DMF:Et_2O, 55ºC, 16 h, 36-44%; (c) $Ni(OAc)_2{\cdot}4H_2O$, $NaBH_4$, ethylenediamine, H_2 (1 atm), EtOH, rt, 2 h, 89-92%; (d) $LiOH{\cdot}H_2O$, THF:H_2O, rt, 16-18 h, 99%.

Next, the glycerol-phosphate moiety was prepared following a synthetic methodology previously set up in the laboratory, consisting on the formation of an intermediate phosphite and its subsequent oxidation.[90] Thus, one-pot reaction of (*S*)-(-)-2,3-bis(benzyloxy)propan-1-ol with di-*tert*-butyl-*N*,*N*-diisopropylphosphoramidite and oxidation with *m*-chloroperbenzoic acid (*m*CPBA) led to phosphate **35**. Removal of the benzyl groups by catalytic hydrogenation yielded diol **36** (Scheme 13).



Scheme 13. Reagents and conditions: (a) i) $iPr_2NP(OtBu)_2$, 1*H*-tetrazole, DCM, rt, 2 h; ii) *m*CPBA, DCM, -30ºC, 90 min, 38%; (b) H_2, 10% Pd(C), EtOH, 60ºC, 99%.

Finally, regioselective esterification reaction between myristoleic or palmitoleic acid or the non-commercial carboxylic acids **32**-**34** and phosphorylated diol **36**, using *N,N'*-dicyclohexylcarbodiimide (DCC) as coupling reagent at low temperature, led to intermediates **37**-**41**, which were then deprotected to obtain the final products **2a-e** with good yields (Scheme 14).



Myristoleic acid (m = 3)
Palmitoleic acid (m = 5)
32 (m = 6)
33 (m = 8)
34 (m = 9)

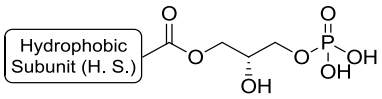
37 (m = 3)
38 (m = 5)
39 (m = 6)
40 (m = 8)
41 (m = 9)

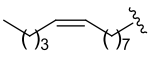
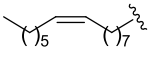
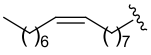
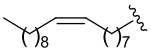
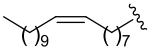
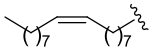
2a-e
(m = 3, 5, 6, 8, 9)

Scheme 14. Reagents and conditions: (a) **36**, DCC, DMAP, DCM, -20ºC to rt, 16 h, 12-49%; (b) TFA, DCM, rt, 4-5 h, 90-99%.

Synthesized compounds **2a**-**e** were tested for their activity at the LPA_1 receptor (Table 5). The most remarkable conclusion that can be drawn from the data obtained is the great influence on activity exerted by the length of the hydrophobic chain. As shown in Table 5, variations in just one methylene unit can turn a compound inactive (for example derivative **2d** shows an activity comparable to LPA whereas **2e** is inactive) or increase its activity almost two-fold, as shown by compounds **2d** (E_{max} = 88%; EC_{50} = 3.6 µM) and **2c** (E_{max} = 202%; EC_{50} = 2.1 µM). These data suggest that the optimal chain is the corresponding to (9*Z*)-hexadec-9-enoic acid (palmitoleic acid), present in compound **2b** (E_{max} = 205%; EC_{50} = 0.45 µM), the most potent derivative within the series and better than the endogenous ligand LPA (E_{max} = 100%; EC_{50} = 0.83 µM).

Table 5. Agonist activities of compounds **2a**-**e** at LPA_1 receptor



Compound	H. S. =	E_{max} (%)[a]	EC_{50} (µM)[b]
2a		127± 1	2.8 ± 0.1
2b		205 ± 9	0.45 ± 0.01
2c		202 ± 1	2.1 ± 0.3
2d		88 ± 2	3.6 ± 0.2
2e		N.E.[c]	-
LPA		100	0.83 ± 0.02

[a] E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [b] For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [c] No effect was observed at the highest concentration of compound tested (10 µM).

2.1.3.2. Derivatives of series **II** containing aromatic rings

Previous results from our group indicate that phenyl group can mimic unsaturated fatty acid chains.[91] In addition, and considering the importance of the length of the chain for activity, the influence of the distance between the aromatic rings and the ester group of the molecule (n) was screened in detail (Figure 15).

In this case, the synthesis involved the preparation of the non-commercial ω-phenylalkanoic acids as well as the conveniently protected phosphorylated glycerol (Figure 15). Regarding the glycerol moiety and searching for synthetic simplicity and high yields, an ethyl group was used for phosphate protection, whose removal conditions should not interfere with the presence of an ester. As for the diol protecting group, an isopropylidene acetal was initially selected.

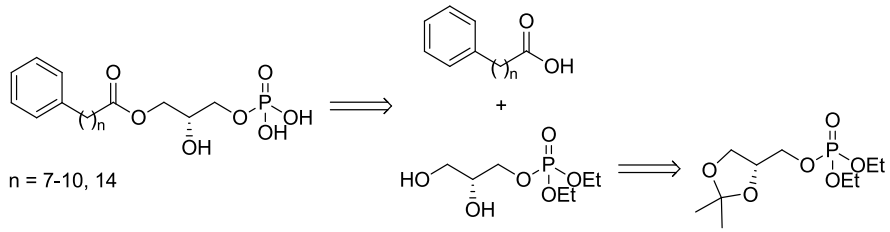
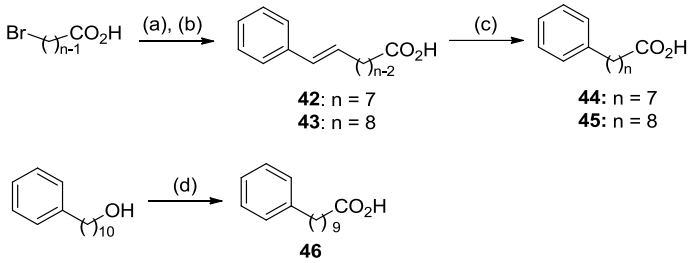


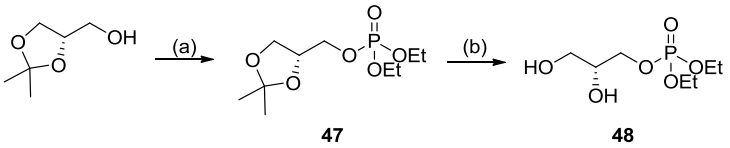
Figure 15. Retrosynthetic analysis for aromatic ring derivatives of series **II**.

Non-commercially available carboxylic acids (n = 7-9) were synthesized as depicted in Scheme 15, either by Wittig reaction between the appropriate bromoacid and benzaldehyde, followed by catalytic hydrogenation, in the case of acids **44** and **45**; or by oxidation of the corresponding alcohol, in the case of acid **46**. 11-Phenylundecanoic acid (n = 10) and 15-phenylpentadecanoic acid (n = 14) were commercially available.



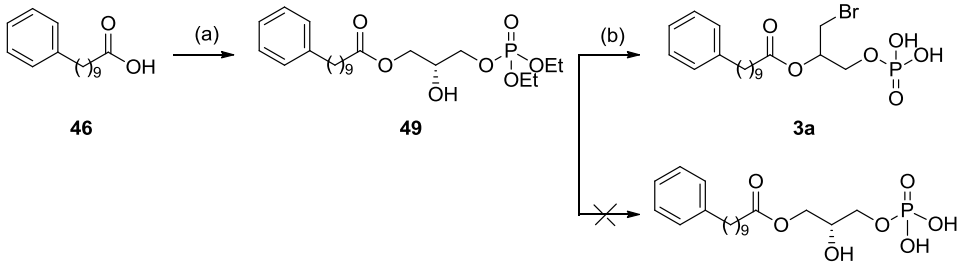
Scheme 15. Reagents and conditions: (a) PPh_3, toluene, reflux, 18 h, 99%; (b) benzaldehyde, lithium bis(trimethylsilyl)amide, THF, -20ºC to rt, 18 h, 61-70%; (c) H_2, 10% Pd(C), EtOH, rt, 81-97%; (d) PDC, DMF, rt, 16 h, 52%.

For the preparation of the phosphorylated glycerol backbone, (*S*)-solketal was treated with diethyl chlorophosphate in the presence of potassium *tert*-butoxide to yield intermediate **47** in very high yield, which after cleavage of the acetal group afforded diol **48** (Scheme 16).



Scheme 16. Reagents and conditions: (a) $ClP(O)(OEt)_2$, KO*t*Bu, DCM, rt, 48 h, 92%; (b) PS-*p*TsOH, CH_3OH, rt, 16 h, 50%.

Once carboxylic acids **44**-**46** and phosphorylated glycerol **48** were prepared, intermediate **46** was used, as first example, to carry out the next step of the proposed synthetic route. Thus, the reaction between **48** and **46** using DCC as coupling reagent led to ester **49**, which was treated with TMSBr as generally described[92] to remove the ethyl groups from the phosphate. However, instead of the expected product, the bromo derivative **3a** was obtained (Scheme 17), which structure was confirmed by nuclear magnetic resonance (NMR) and high pressure liquid chromatography coupled to mass spectrometry (HPLC-MS). The reaction mechanism proposed for the formation of compound **3a** and the determination of its absolute configuration will be discussed later.

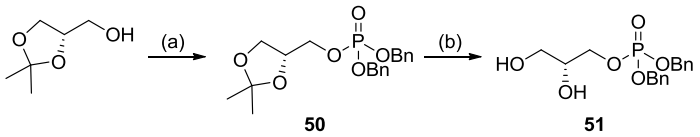


Scheme 17. Reagents and conditions: (a) **48**, DCC, DMAP, -20ºC to rt, 18 h, 30%; (b) i) TMSBr, DCM, rt, 4 h; ii) $MeOH/H_2O$, rt, 1h, 90%.

Although different deprotection conditions using TMSBr were assayed for compound **49**, it was not possible to isolate the desired product. Starting material was used thoroughly dried, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide was employed as additive, and the reaction times were reduced, but mixtures of starting material, desired product and brominated product, which could not be separated by chromatography, were obtained in all cases.

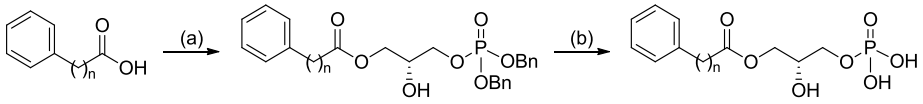
A benzyl group was then considered as an alternative phosphate protecting group in order to avoid the formation of compound **3a**. Hence, dibenzyl phosphate glycerol

derivative **51** was obtained from (*S*)-solketal using the appropriate phosphoramidite reagent according to the methodology previously used, followed by deprotection of the diol (Scheme 18).



Scheme 18. Reagents and conditions: (a) i) *i*Pr_2NP(OBn)$_2$, 1*H*-tetrazole, DCM, rt, 2 h; ii) *m*CPBA, DCM, -30ºC, 90 min, 76%; (b) PS-*p*TsOH, CH_3OH, rt, 16 h, 65%.

The regioselective esterification reaction between diol **51** and the corresponding ω-phenylalkanoic acids (n = 7-10, 14) allowed to obtain the esters **52**-**56**, which after elimination of the benzyl groups by catalytic hydrogenation afforded the desired final compounds **2f-j** (Scheme 19).



44: n = 7
45: n = 8
46: n = 9
11-Phenylundecanoic acid: n = 10
15-Phenylpentadecanoic acid: n =14

52: n = 7
53: n = 8
54: n = 9
55: n = 10
56: n =14

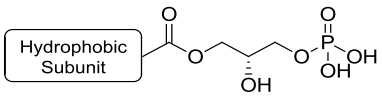
2f: n = 7
2g: n = 8
2h: n = 9
2i: n = 10
2j: n = 14

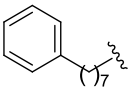
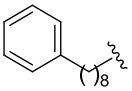
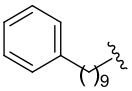
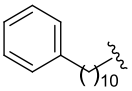
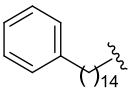
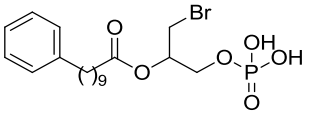
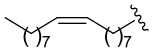
Scheme 19. Reagents and conditions: (a) **51**, DCC, DMAP, DCM, -20ºC to rt, 16 h, 18-58%; (b) H_2, 10% Pd(C), EtOH, rt, 80-99%.

Synthesized compounds **2f**-**j** were tested for activity at LPA_1 receptor and the results are shown in Table 6. Although compound **3a** bears a different glycerol phosphate scaffold, it was also tested, taken also into account that compounds containing a bromine in the polar head have been described as good agonists or antagonists for LPA receptors.[93]

Again, the obtained data (Table 6) point out the importance of the length of the hydrophobic chain of the compound in the activity at LPA_1 receptor, as small changes in the number of methylene units dramatically affects E_{max} and EC_{50} values. For example, compound **2f**, with n = 7, is inactive, whereas derivative **2g**, with n = 8, is almost as active as LPA. In addition, it must be highlighted that compound **3a**, with a different polar head group, exhibits a very good activity at LPA_1 receptor. Among these derivatives, compounds **2h** and **3a**, with E_{max} > 100% and EC_{50} values of 0.5 and 0.24 μM, respectively, stand out.

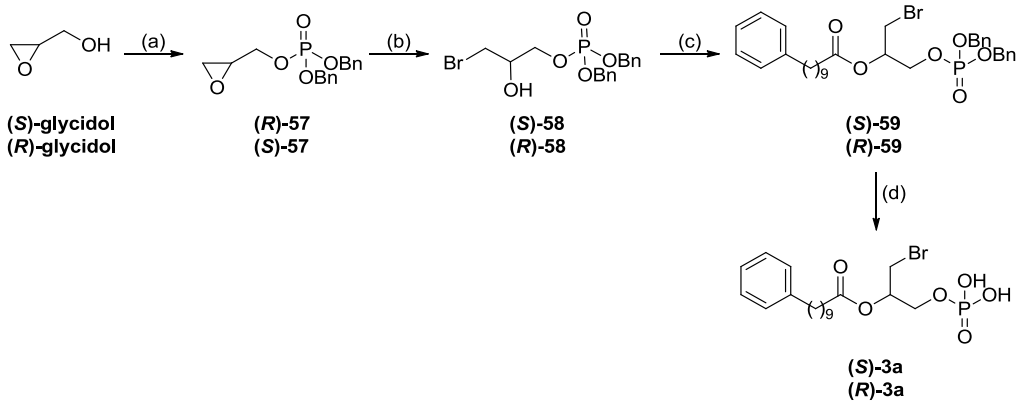
Table 6. Agonist activities of compounds **2f**-**j** and **3a** at LPA_1 receptor



Compound	Hydrophobic Subunit	E_{max} (%)[a]	EC_{50} (μM)[b]
2f		N. E.[c]	-
2g		74 ± 4	2.1 ± 0.3
2h		112 ± 3	0.5 ± 0.1
2i		135 ± 31	3.2 ± 0.5
2j		N. E.	-
3a		118 ± 24	0.24 ± 0.09
LPA		100	0.83 ± 0.02

[a]E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [b]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [c]No effect was observed at the highest concentration of compound tested (10 μM).

Taking into account that the best agonist identified up to this moment was unexpected product **3a**, and in order to establish the configuration of its chiral center, we decided to synthesize both enantiomers of this molecule using a chiral pool strategy. Thus, compounds **(*S*)-** and **(*R*)-3a** were obtained from the corresponding *S* or *R* form of oxiran-2-ylmethanol (glycidol), respectively, which was phosphorylated following the usual conditions. Then, opening of the oxirane ring of intermediates **(*R*)-** and **(*S*)-57** with tetrabutylammonium bromide (TBABr) in the presence of TFA yielded derivatives **(*S*)-** and **(*R*)-58**, which were coupled with acid **46**. Subsequent catalytic hydrogenation of the benzyl groups gave the desired final compounds **(*S*)-** and **(*R*)-3a** (Scheme 20).



Scheme 20. Reagents and conditions: (a) i) *i*$Pr_2NP(OBn)_2$, 1*H*-tetrazole, DCM, rt, 2 h; ii) *m*CPBA, DCM, -30ºC, 90 min, 76-95%; (b) TBABr, TFA, $CHCl_3$, rt, 10 min, 89-99%; (c) **46**, DCC, DMAP, DCM, rt, 18 h, 68-73%; (d) H_2, 10% Pd(C), EtOH, rt, 97-99%.

The determination of the specific optical rotation of both enantiomers of **3a** ($[\alpha]_D^{20}$ **(*S*)-3a** = +4.8, $[\alpha]_D^{20}$ **(*R*)-3a** = -4.6, c = 0.50 methanol) confirmed that the compound obtained during the phosphate deprotection reaction (see Scheme 17), which had a positive optical rotation value, was the enantiomer *S* ($[\alpha]_D^{20}$ **3a** = +4.9, c = 0.50 methanol). The formation of **(*S*)-3a** in this reaction could be explained by migration of the acyl chain to the secondary hydroxy group, and subquent substitution of the primary hydroxy group by the bromide present in the reaction media (Figure 16). Intramolecular acyl chain migration, facilitated by both acidic and basic conditions, is a well known phenomenon of LPs, specially in the case of 2-acyl-*sn*-glycerol-3-phosphate species, which are very labile under physiological conditions and easily isomerize to 1-acyl derivatives.[94]

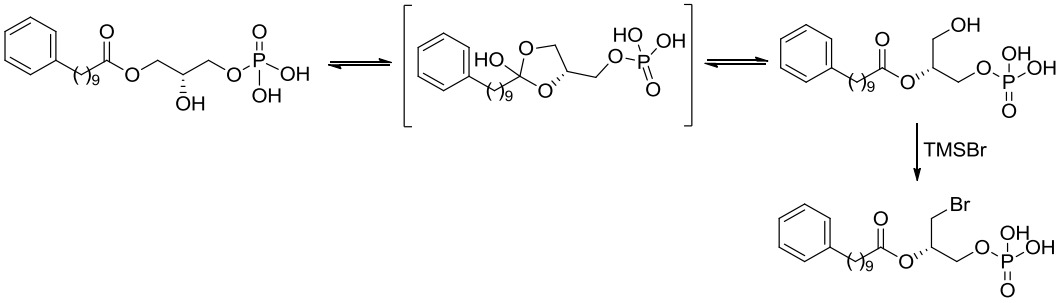
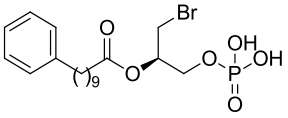
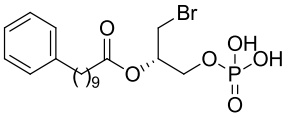
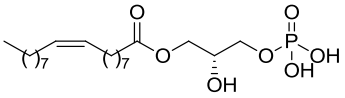


Figure 16. Proposed mechanism for the formation of compound **(*S*)-3a**.

At this point, the effect of the stereocenter in the ligand activity was considered, and the assessment of the agonist activity of the *R* enantiomer of **3a** revealed an enantiomer preference, as *R* isomer is much less active than the *S* one, showing an increase of two orders of magnitude in the EC_{50} value (Table 7).

Table 7. Agonist activitity of compounds **(*R*)-** and **(*S*)-3a** at LPA_1 receptor

Compound	Structure	E_{max} (%)[a]	EC_{50} (μM)[b]
(*R*)-3a		32 ± 3	26 ± 5
(*S*)-3a		118 ± 24	0.24 ± 0.09
LPA		100	0.83 ± 0.02

[a]E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [b]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate.

2.1.3.3. Synthesis of analogues of **(*S*)-3a**

Considering the excellent activity of compound **(*S*)-3a**, which turned out to be the best LPA_1 receptor agonist among all the synthesized compounds so far (E_{max} = 118%, EC_{50} = 0.24 μM), additional structural exploration was extended around this new scaffold. In particular, it was analysed whether changes in the length of the methylenic chain would affect its activity in the same degree observed for compounds **2f**-**2j** of series **II**. In addition, the replacement of the hydrophobic unit by the oleoyl chain was studied, in order to see if it would involved an increase in activity. Hence, compounds **3b**-**d** (Figure 17) were synthesized.

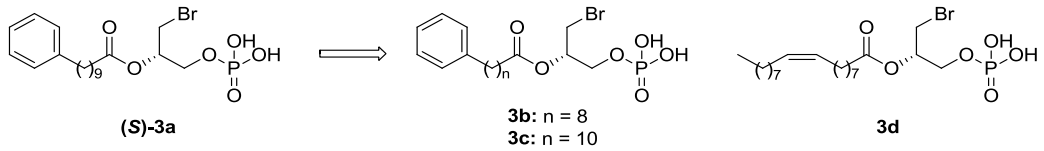
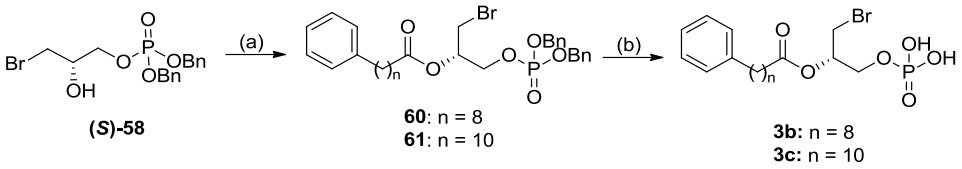


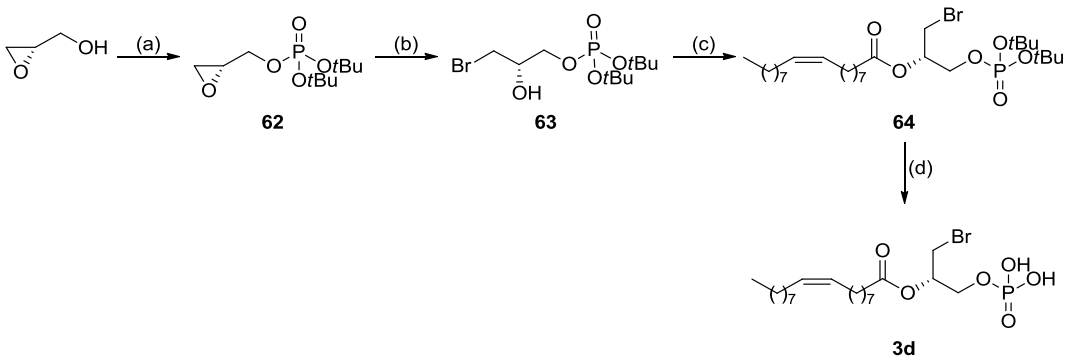
Figure 17. Designed derivatives of **(*S*)-3a**.

Phenyl-containing derivatives **3b** and **3c** were obtained from **(*S*)-58**, which was coupled with the corresponding ω-phenylalkanoic acids. Subsequent catalytic hydrogenation of the benzyl groups gave the desired final compounds **3b**, **c** (Scheme 21).



Scheme 21. Reagents and conditions: (a) **45** or 11-phenylundecanoic acid, DCC, DMAP, DCM, rt, 5 h, 55-96%; (b) H_2, 10% Pd(C), EtOH, rt, 72-80%.

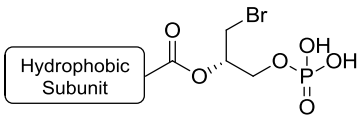
A similar synthetic route, but employing *tert*-butyl instead of benzyl as protecting group for the phosphate, due to the presence of the double bond in the oleate moiety, allowed to obtain compound **3d** starting from (*S*)-glycidol (Scheme 22).

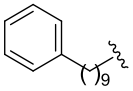
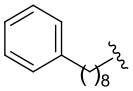
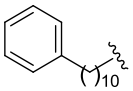
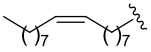
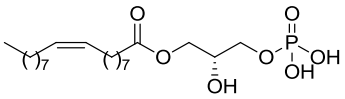


Scheme 22. Reagents and conditions: (a) i) *i*Pr_2NP(*t*Bu)$_2$, 1*H*-tetrazole, DCM, rt, 2 h; ii) *m*CPBA, DCM, -30ºC, 90 min, 52%; (b) TBABr, TFA, $CHCl_3$, rt, 10 min, 95%; (c) oleoyl chloride, pyridine, rt, 18 h, 15%; (d) TFA, DCM, rt, 5 h, 81%.

Activity of these new compounds at the LPA_1 receptor was determined (Table 8). The obtained results showed that only oleoyl derivative **3d** was able to activate the receptor, although it did not reach neither LPA nor **(*S*)-3a** values.

Table 8. Agonist activities of compounds **(*S*)-3a** and **3b**-**d** at LPA_1 receptor



Compound	Hydrophobic Subunit	E_{max} (%)[a]	EC_{50} (μM)[b]
(*S*)-3a		118 ± 24	0.24 ± 0.09
3b		N. E.[c]	-
3c		N. E.	-
3d		39 ± 3	3.2 ± 0.4
LPA		100	0.83 ± 0.02

[a]E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [b]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [c]No effect was observed at the highest concentration of compound tested (10 μM).

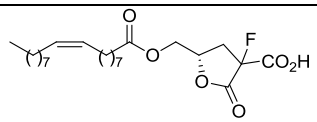
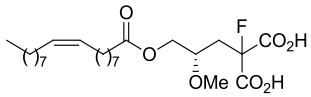
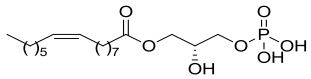
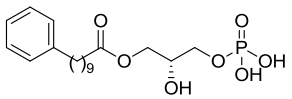
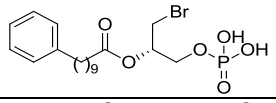
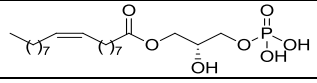
2.1.4. Selectivity of the identified LPA_1 agonists over LPA_2 and LPA_3 receptors

The purpose of present work is the development of potent and selective LPA_1 receptor agonists. Taking into account the high sequence homology among the LPA receptors, especially LPA_{1-3}, the next step is to determine the selectivity of the identified agonists towards LPA_2 and LPA_3 receptors.

Hence, cell lines stably overexpressing LPA_2 and LPA_3 receptor subtypes were generated. A B103 neuroblastoma cell line, which does not express LPA receptors intrinsically,[95] was chosen for its transfection with the corresponding plasmid containing LPA_2 and LPA_3 receptors fused with the enhanced green fluorescent protein (EGFP). B103 cells expressing high levels of EGFP, and thus of the desired receptor, were isolated by fluorescence-activated cell sorting (FACS).

The best agonist of each series (**1c**, **1g**, **2b**, **2h** and **(*S*)-3a**, see Tables 3-6) was selected and screened for activity at these cell lines. The obtained results (Table 9) showed that compounds **1c**, **1g** and **(*S*)-3a** are selective for LPA_1 versus LPA_2 and LPA_3 receptors. Compound **2b** can also be considered selective, given that it is 50-fold less active at LPA_3 receptor than at LPA_1 receptor. Lack of activity at LPA_3 receptor is especially important, given that the activation of this receptor is implicated in the production of high LPA levels after peripheral nerve damage, responsible for initiation and maintenance of NP (see Introduction pag. 6).

Table 9. Agonist activity of compounds at LPA_{1-3} receptor

		EC_{50} (μM)[a]		
	Compound	**LPA_1 receptor**	**LPA_2 receptor**	**LPA_3 receptor**
1c		1.7 ± 0.2	N. E. [b]	N. E.
1g		6 ± 1	N. E.	N. E.
2b		0.45 ± 0.01	N. E.	21.6 ± 5
2h		0.5 ± 0.1	5.3 ± 0.6	N. E.
(*S*)-3a		0.24 ± 0.09	N. E.	N. E.
LPA		0.83 ± 0.02	1.3 ± 0.5	3.4 ± 0.8

[a]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [b]No effect was observed at the highest concentration of compound tested (10 μM).

2.1.5. Combination of the acid and hydrophobic subunits

Among all the synthesized compounds, derivatives **1c**, **1g**, **2b** and **(*S*)-3a** were selected as the most active and selective agonists at the LPA_1 receptor (Figure 18). They present EC_{50} values between 0.24 and 6 μM, comparable or even better than the endogenous ligand LPA (EC_{50} = 0.83 μM). This suggests that the phosphate glycerol backbone of LPA can be mimicked by other acid moieties such as (5*S*)-3-fluoro-5-(hydroxymethyl)-2-oxotetrahydrofuran-3-carboxylic acid (present in **1c**), fluoro[(2*S*)-3-hydroxy-2-methoxypropyl]malonic acid (present in **1g**) and (2*S*)-3-bromo-2-hydroxypropyl dihydrogen phosphate (present in **(*S*)-3a**). In addition to the oleoyl chain of LPA, the LPA_1 receptor recognizes shorter chains such as those derived from palmitoleic acid (present in **2b**) and 10-phenyldecanoic acid (present in **(*S*)-3a**) (Figure 18).

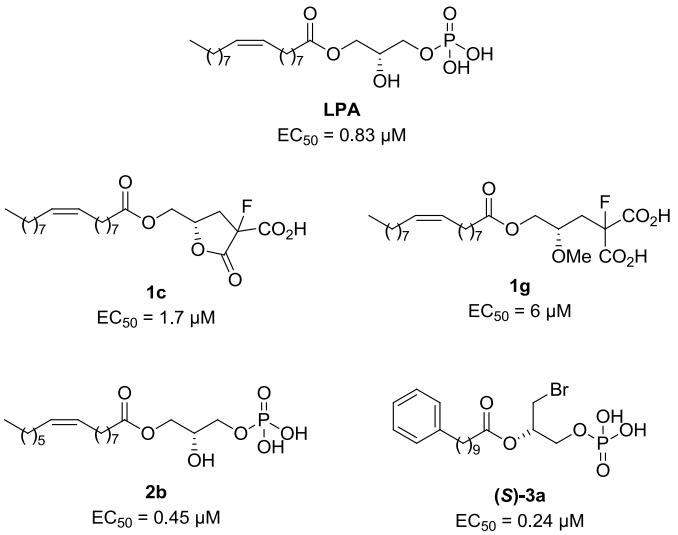


Figure 18. Compounds with activity and selectivity at the LPA_1 receptor.

With these results in hand, we combined the hydrophobic chains present in these compounds with the polar heads that seemed to be able to mimic the LPA phosphate group. Hence, the fluorinated lactone and malonic acid, and the bromo-phosphate moieties present in derivatives **1c**, **1g** and **(*S*)-3a** were selected as polar heads. Regarding the hydrophobic subunit, oleoyl (present in **1c** and **1g**), palmitoleoyl (present in **2b**) and 10-phenyldecanoyl chains [present in **(*S*)-3a**] were chosen. The combination of these fragments led to products **1c**, **1g**, **(*S*)-3a**, and **3d** (which were previously synthesized) and new derivatives **4a**-**e** which were synthesized and tested as agonists of the LPA_1 receptor (Figure 19).

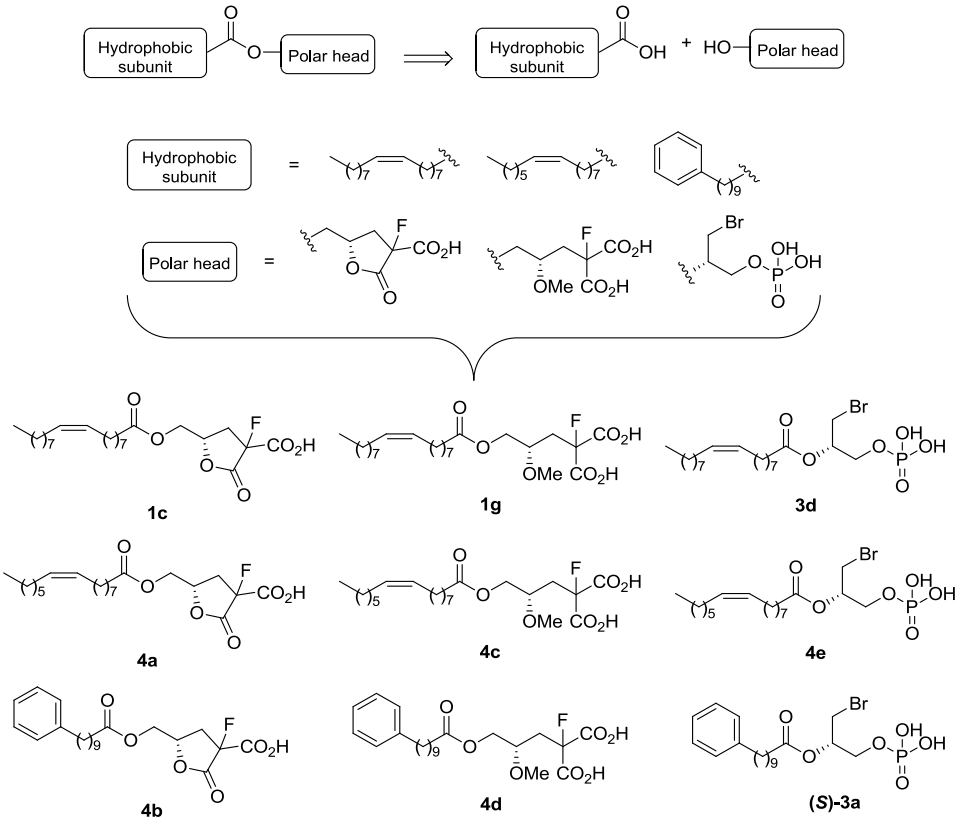
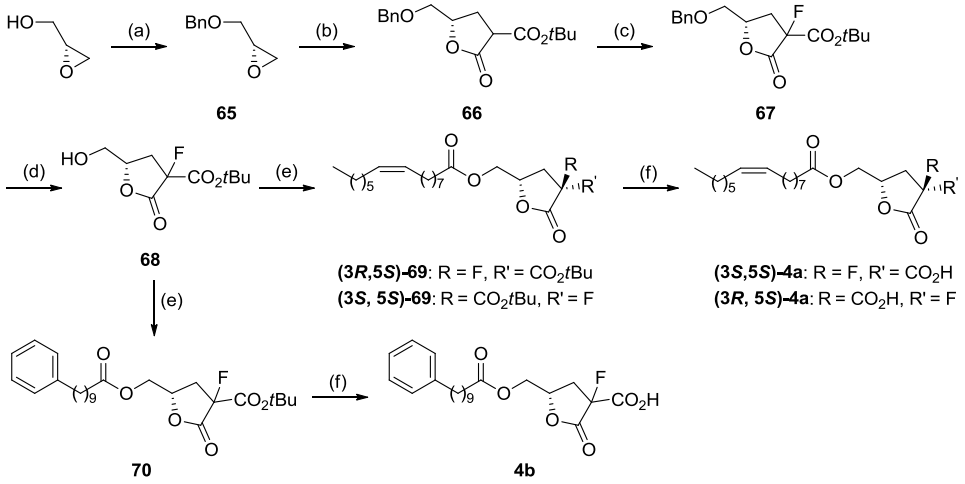


Figure 19. Combination of the acid and hydrophobic subunits.

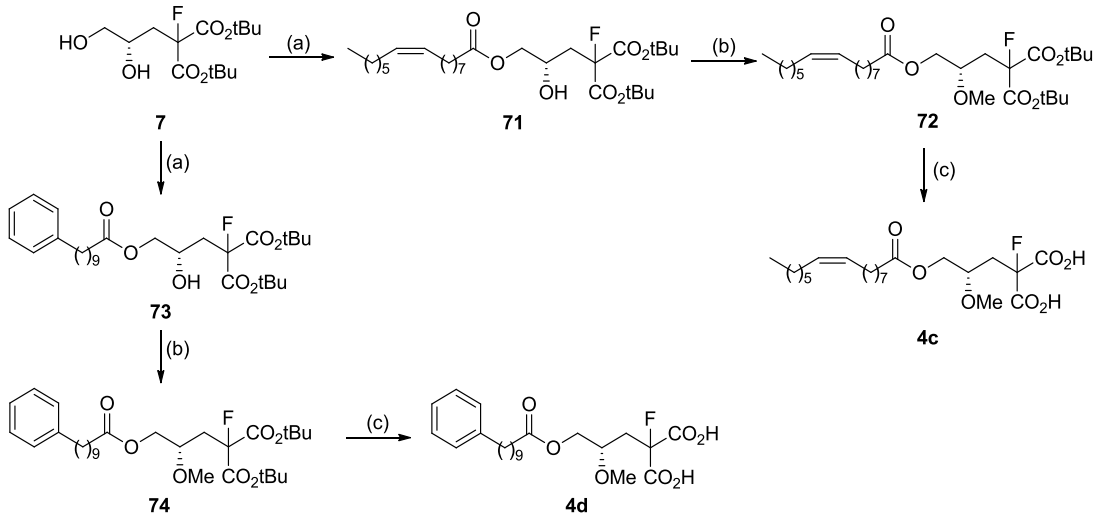
The synthesis of lactones **4a**,**b** started with benzylation of (*R*)-glycidol, followed by oxirane opening reaction with di-*tert*-butyl malonate in basic media with concomitant intramolecular cyclization to give lactone **66**, as a mixture of diastereoisomers. Fluorination reaction under usual conditions led to derivative **67**, whose catalytic hydrogenation yielded alcohol **68**. The esterification reaction between **68** and palmitoleic acid afforded intermediate **69**, which chromatographic purification allowed the separation of the two diastereoisomers. Final deprotection of both diastereoisomers led to compounds **(3*R*,5*S*)-** and **(3*S*,5*S*)-4a** (Scheme 23). The

absolute configuration of the chiral carbon bonded to the fluorine atom was determined on the basis of NOE experiments and the values of the (H,H) and (H,F) coupling constants in ^{1}H- and ^{19}F-NMR spectra. In the case of esterification with 10-phenyldecanoic acid **46**, derivative **4b** was obtained after treatment with TFA as 1:1 mixture of diastereoisomers (Scheme 23).



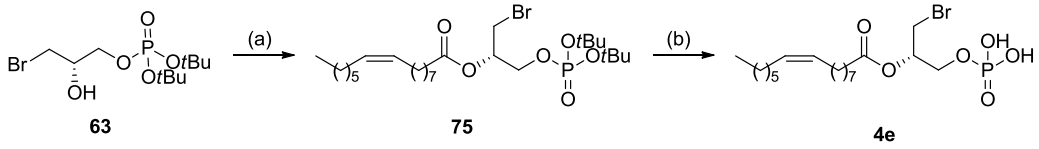
Scheme 23. Reagents and conditions: (a) BnBr, NaH, DMF, 0ºC to rt, 18 h, 99%; (b) di-*tert*-butyl malonate, NaH, DMF:THF, 0ºC to 80ºC, 6 h, 62%; (c) Selectfluor®, NaH, DMF:THF, 0ºC to rt, 48 h, 99%; (d) H_2, 10% Pd(C), 60ºC, 87%; (e) palmitoleic acid or **46**, DCC, DMAP, rt, 18 h, 25-35%; (f) TFA, DCM, rt, 17 h, 69-90%.

Malonic acid derivatives **4c**,**d** were prepared by regioselective coupling of the corresponding carboxylic acid and diol **7**. Methylation of the hydroxy group present in the resulting esters **71** and **73** with trimethylsilyldiazomethane followed by removal of the *tert*-butyl groups yielded the desired compounds **4c**,**d** (Scheme 24).



Scheme 24. Reagents and conditions: (a) Palmitoleic acid or **46**, DCC, DMAP, DCM, -20ºC to rt, 17 h, 44-75%; (b) trimethylsilyldiazomethane, HBF_4, DCM, 0ºC, 90 min, 27-30%; (c) TFA, DCM, rt, 18 h, 68-93%.

Final product **4e** was prepared by esterification reaction between palmitoleic acid and bromo alcohol **63**, followed by phosphate group deprotection using TFA (Scheme 25).



Scheme 25. Reagents and conditions: (a) Palmitoleic acid, DCC, DMAP, DCM, rt, 24 h, 55%; (b) TFA, DCM, rt, 5 h, 93%.

Compounds **4a**-**e** were assayed for their activity at LPA_1 receptor and the results are shown in Figure 20, where previously tested compounds **1c**, **1g**, **(*S*)-3a** and **3d** have been included of comparison (Figure 20). The combination process has highlighted that replacement of the phosphate moiety is tolerated only in the presence of oleoyl chain as hydrophobic subunit (compounds **1c**, **1g** and **3d**).

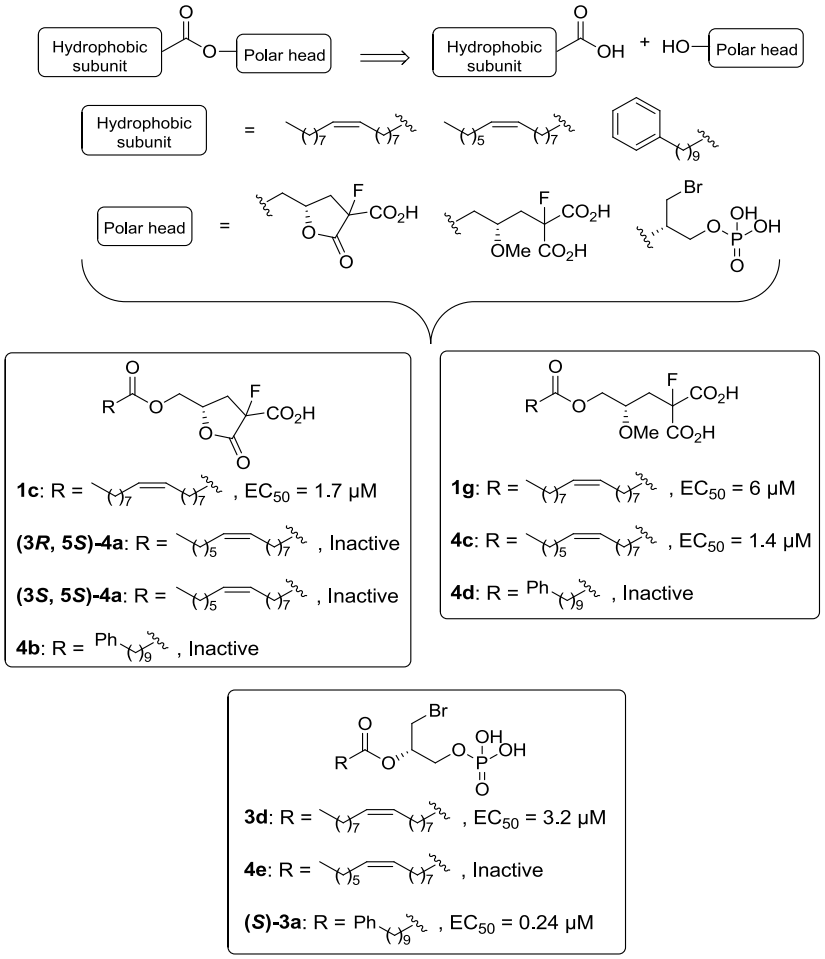
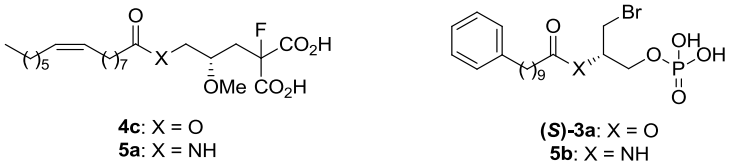


Figure 20. Agonist activity of compounds **1c**, **1g**, **(*S*)-3a**, **3d** and **4a**-**e** at LPA_1 receptor.

In summary, the two series proposed for mimicking the hydrophobic chain and the polar head of the endogenous ligand LPA, together with the combination process, have yielded new scaffolds capable to activate the receptor. Among them, derivatives **(*S*)-3a** and **4c** stand out as agonists structurally different from the endogenous ligand LPA with excellent activity values. Besides, the screening at LPA_2 and LPA_3 receptors showed that compound **4c** is also selective for LPA_1 receptor. Hence, these two compounds were selected for further optimization process.

2.1.6. Structural modifications of compounds ***(S)*-3a** and **4c**

The presence of an amide group in a drug molecule is usually associated to better chemical and metabolic stability. Thus, the synthesis of amides **5a** and **5b**, derived from compounds **4c** and **(*S*)-3a**, respectively, were proposed in order to obtain LPA agonists with improved stability properties (Figure 21).

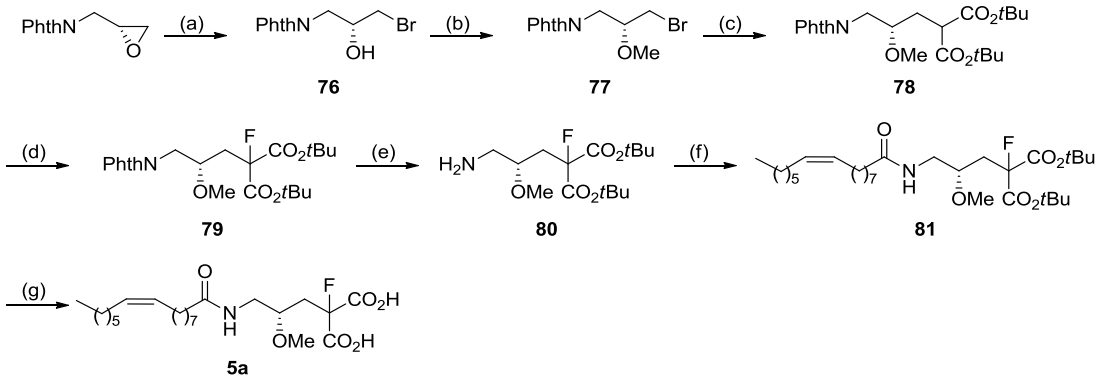


4c: X = O
5a: X = NH

(*S*)-3a: X = O
5b: X = NH

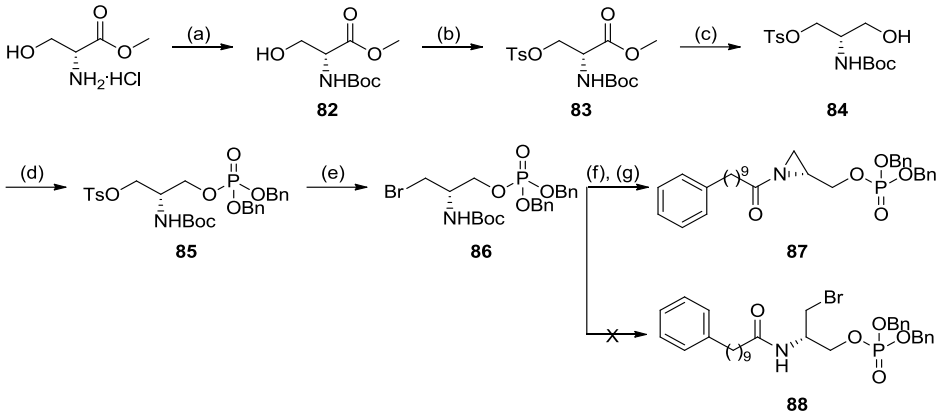
Figure 21. Amides **5a** and **5b**.

The synthesis of compound **5a** started with the ring-opening reaction of the oxirane present in (*R*)-(−)-*N*-(2,3-epoxypropyl)phthalimide with hydrobromic acid followed by methylation of the resulting hydroxy group with trimethylsilyldiazomethane to obtain intermediate **77**, which was alkylated with di-*tert*-butyl malonate to afford derivative **78**. This compound was treated with Selectfluor® to yield the fluorinated analogue **79**. Next, deprotection reaction with hydrazine led to amine **80**, which was coupled with palmitoleic acid to obtain final compound **5a** after treatment with TFA (Scheme 26).



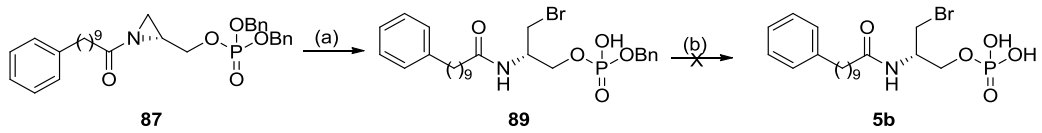
Scheme 26. Reagents and conditions: (a) HBr, $CHCl_3$, 0°C, 30 min, 96%; (b) HBF_4, $TMSCHN_2$, DCM, 0°C, 2 h, 62%; (c) di-*tert*-butyl malonate, NaH, NaI, DMF, rt, 12 h, 54%; (d) Selectfluor®, NaH, DMF:THF, 0ºC to rt, 48 h, 96%; (e) N_2H_4, EtOH, rt, 12 h, 66%; (f) palmitoleic acid, EDC, HOBt, DMF, rt, 12 h, 37%; (g) TFA, DCM, rt, 12 h, 99%.

In the case of compound **5b**, the synthesis started with the amino protection of the methyl ester of D-serine with the *tert*-butyloxycarbonyl group (Boc), followed by transformation of the hydroxy group into the corresponding tosylate to obtain derivative **83**. Reduction of the methyl ester with $NaBH_4$ yielded alcohol **84**, which was phosphorylated under usual conditions. Next, nucleophilic substitution using lithium bromide led to intermediate **86**. At this point, the Boc group was removed by treatment with TFA, and condensation of the free amine with the *N*-hydroxysuccinimyl ester of 10-phenyldecanoic acid (**46**) was carried out in order to obtain intermediate amide **88**, precursor of final compound **5b**. However, the coupling reaction did not provide the desired compound and aziridine **87** was formed, as a consequence of an intramolecular cyclization (Scheme 27).



Scheme 27. Reagents and conditions: (a) Boc_2O, Et_3N, THF, MW, 100°C, 8 min, 99%; (b) *p*TSCl, pyridine, DCM, 0°C to rt, 24 h, 49%; (c) $NaBH_4$, EtOH, -5°C, 1 h, 96%; (d) i) $iPr_2NP(OBn)_2$, 1*H*-tetrazole, DCM, rt, 2 h; ii) *m*CPBA, DCM, -30ºC, 90 min, 38%; (e) LiBr, acetone, reflux, 12 h, 79%; (f) TFA, DCM, rt, 1 h, 99%; (g) *N*-hydroxysuccinimyl ester of **46**, Et_3N, DCM, rt, 12 h, 48%.

In view of this result, a ring opening reaction of aziridine **87** with bromide was considered as an alternative route to obtain the β-bromoamide **88**.[96] Thus, **87** was treated with magnesium bromide at room temperature, yielding compound **89**, in which, besides the opening of the aziridine, the removal of one of the benzyl protecting groups was observed. This new intermediate was directly subjected to catalytic hydrogenation for complete deprotection of the phosphate (Scheme 28). Unfortunately, compound **5b** could not be isolated, and a complex mixture of unidentified products was obtained instead, which could be explained by a low intrinsic stability of target compound.



Scheme 28. Reagents and conditions: (a) $MgBr_2 \cdot OEt_2$, Et_2O, rt, 4 h, 81%; (b) H_2, 10% Pd(C), EtOH, rt.

Compound **5a** was tested for its activity at LPA_1, but it was not able to activate the receptor, showing that the replacement of the ester group by an amide is detrimental for the activity.

In summary, considering that potent activation of the LPA_1 receptor is needed in order to induce its desensitization and internalization, compound **(*S*)-3a**, with an excellent agonist activity and selectivity versus LPA_2 and LPA_3 receptors, was selected as the best candidate to move forward in our study of the therapeutic potential of LPA_1 agonists for the treatment of NP.

2.2. Biological characterization of compound (*S*)-3a

To further characterize the biological potential of **(*S*)-3a**, we carried out a set of additional experiments aimed at confirming that **(*S*)-3a** bound directly LPA_1 receptor and studying whether, after receptor binding and activation, it induced internalization and desensitization.

2.2.1. LPA_1 receptor binding affinity of compound **(*S*)-3a**

The biological response produced by a drug is related to both the efficacy and the binding affinity for its molecular target. Therefore, the affinity of agonist **(*S*)-3a** for LPA_1 receptor was determined in order to complete the pharmacological characterization of the compound. In the case of LPA receptors, the use of radioligand is not straightforward due to the high non-specific binding, that leads to low signal to noise ratio. Hence, this experiment was carried out using a technique named back-scattering interferometry (BSI),[97,98] during a predoctoral stay at Prof. Jerold Chun Lab at The Scripps Research Institute (La Jolla, California). BSI monitors changes in refractive index (RI) to detect molecular interactions in a label-free, free-solution manner. This shift in RI is a result of changes in molecular structure, dipole moment, polarizability, conformation, and solvation that occur during the interaction of the proteins with their ligands.

Thus, BSI can be used to quantify binding affinities by detecting changes in the RI of mixtures of protein and ligand that have been incubated to achieve equilibrium. In this case, ligand binding to membrane homogenates containing LPA_1 receptor was accomplished by incubating a solution of a fixed protein concentration with varying concentrations of compound **(*S*)-3a**. The difference between the signals of protein-ligand complex and free protein solutions was plotted versus ligand concentration to obtain a saturation binding isotherm (Figure 22), which was fitted to a square hyperbolic function using GraphPad Prism software to determine the dissociation constant (K_D) value (Table 10). Binding affinity of LPA to LPA_1 receptor was determined as positive control, whereas the plasmid control without any receptor (VEC) was used as negative control (Figure 22).

The obtained K_D value indicated a high binding affinity of **(*S*)-3a** for LPA_1 receptor (K_D = 19.9 nM), comparable to that obtained of the endogenous ligand LPA (K_D = 5.6 nM) (Table 10).

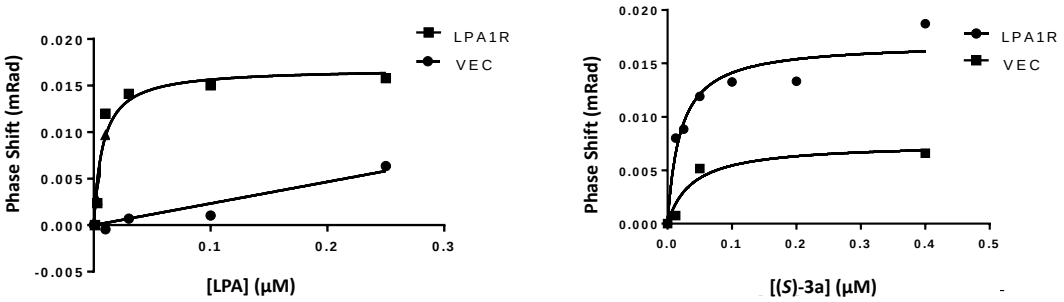


Figure 22. Representative plots of BSI signal versus ligand concentration for the determination of affinity constant. Each data point represents the average of at least four independent measurements. Membranes from cells transfected with the empty plasmid -without any receptor (VEC)- were used as negative control.

Table 10. Affinity constants (K_D) determined by BSI at LPA_1 receptor

Compound	K_D (nM)[a]	R^2 (%)[b]
(*S*)-3a	19.9	93
LPA	5.6	92

[a] K_D values are the means from two or three independent experiments performed in triplicate. The s.e.m. is in all cases within a 10% of the mean value. [b] A goodness of fit (R^2) greater than 90% is considered adequate.

2.2.2. Receptor internalization

B103 neuroblastoma cells overexpressing LPA_1 receptor fused to EGFP were exposed to compound **(*S*)-3a** at concentrations ranging from 0.1 µM to 10 µM. LPA was employed as positive control and 0.1% fatty acid free bovin serum albumin (FAF BSA) as negative control. After fixing and staining the cells, images were acquired with a confocal microscope. Compound **(*S*)-3a** was able to induce internalization of the receptor at all the concentrations assayed in a similar manner to LPA. Representative image at 1 µM concentration of **(*S*)-3a** is shown in Figure 23.

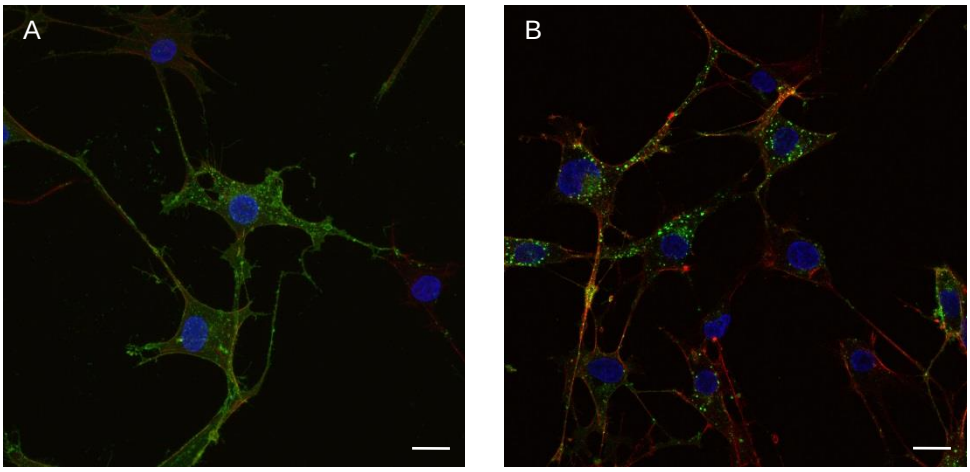


Figure 23. Receptor internalization of LPA_1 receptor by agonist stimulation. Cells were treated with compound **(*S*)-3a** (1 µM) (A) or with LPA, 1 µM (B). Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and phalloydin for cell morphology. Samples were imaged under the same conditions by using a Zeiss fluorescence confocal microscope (bars 10 µm).

2.2.3. Receptor desensitization

Next, we determined the capacity of compound **(*S*)-3a** to induce desensitization in DRG neurons and accordingly block the signal trasduction involved in the pain transmission. Thus, calcium influx and action potential in sensory neurons were measured in order to evaluate the capacity of **(*S*)-3a** to induce desensitization. These experiments were done in collaboration with Prof. Antonio Vicente Ferrer Montiel, at Universidad Miguel Hernández de Elche (Alicante, Spain). Our data show that after a first administration, compound **(*S*)-3a** activates the LPA_1 receptor in DRG sensory neurons in primary culture in a similar manner to LPA (Figure 24). However, a second administration of the agonist at 4 minutes is not able to trigger activation, reflecting the desensitization of the receptor (Figure 24A). A similar behavior was observed for **(*S*)-3a** under the same conditions (Figure 24B). Neurons were exposed to 500 nM of the agonist capsaicin (Caps) at the end of the recording to measure TRPV1 receptor sensitivity of the neurons exposed to LPA or **(*S*)-3a**.

Furthermore, action potential of DRG neurons was affected when treated either with 10 µM LPA or **(*S*)-3a** (Figure 25), confirming the action of this agonist in sensory neurons in primary culture.

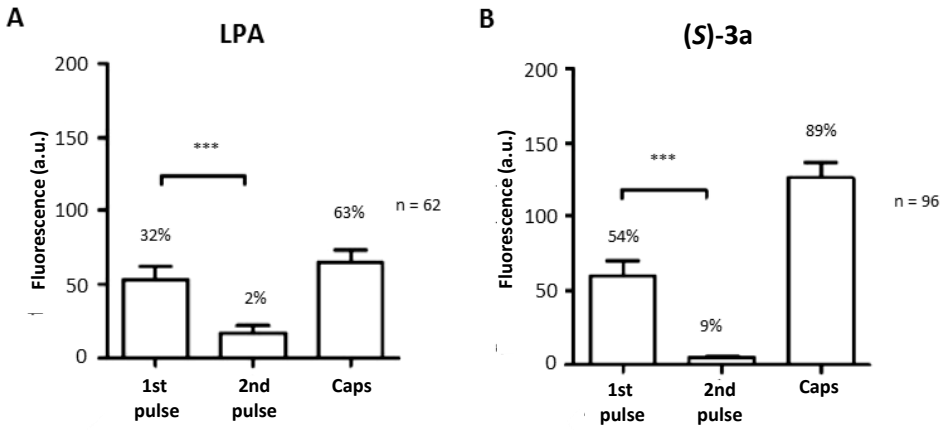


Figure 24. Measurement of calcium-influx peak intensity upon two repetitive applications of 10 µM LPA (A) or **(*S*)-3a** (B) and one of 500 nM capsaicin (Caps) in DRG neurons. Data represent fluorescence expressed as mean ± s.e.m. First pulse (1st) was compared to the second (2nd) by t-test paired values, ***p<0.001. Number above columns represent percentage of responding cells to that stimulus, n = total registered neurons.

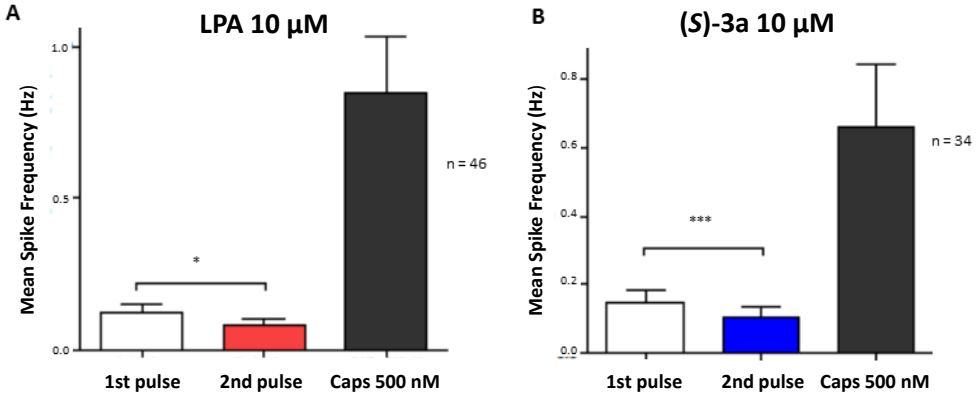


Figure 25. Effect of 10 µM LPA (A) or **(*S*)-3a** (B) on the neuronal firing activity on DRG neurons. LPA and **(*S*)-3a** were applied in two consecutive pulses (1st and 2nd pulse). Data represent mean spike frequency (Hz) and are expressed as mean ± s.e.m. Number of cultures ≥ 2. Comparison of 1st and 2nd pulse was performed by t-test paired values. *p<0.05; ***p<0.001

All in sum, the data obtained up to this moment confirm the capacity of **(*S*)-3a** to promote the internalization of LPA_1 receptor and its desensitization in sensory neurons, which are involved in pain transmission. Hence, all these results have prompted us to consider **(*S*)-3a** a promising candidate for the treatment of NP and to study its in vivo efficacy in a mouse model of NP, experiments that are currently ongoing in collaboration with Prof. Fernando Rodríguez de Fonseca, at Instituto de Investigación Biomédica de Málaga (Spain).

2.3. Design and synthesis of new antagonists for the LPA_1 and LPA_2 receptors

The discovery that LPA_1 and LPA_2 receptors contribute to the etiology of different disorders has prompted the development of selective antagonists for these receptors for pharmacological evaluation. The disclosed antagonists include lipid-like molecules that share structural similarity to LPA, and non-lipid chemical entities.[99-101] The LPA analogues that antagonize LPA_1 or LPA_2 are generally not selective and lack the appropriate pharmacokinetic profiles to be useful for evaluation in clinical studies. For these reasons, broad emphasis has been placed during recent years on the design and discovery of non-lipid LPA receptor antagonists.

Among the different potential therapeutic indications, SCI is currently receiving much interest. It has been recently described that activation of LPA_1 and LPA_2 receptors is detrimental for secondary damage after SCI, suggesting that LPA_1 and LPA_2 antagonists might be useful tools not only to elucidate the specific mechanisms implicated in this pathology, but also for its therapeutic treatment. In order to discover new antagonists of these receptors we performed a virtual screening (VS) of the ZINC library of compounds against the LPA_1 receptor, which 3D structure has just been described in complex with the selective antagonist ONO-9780307 (Figure 26).[102]

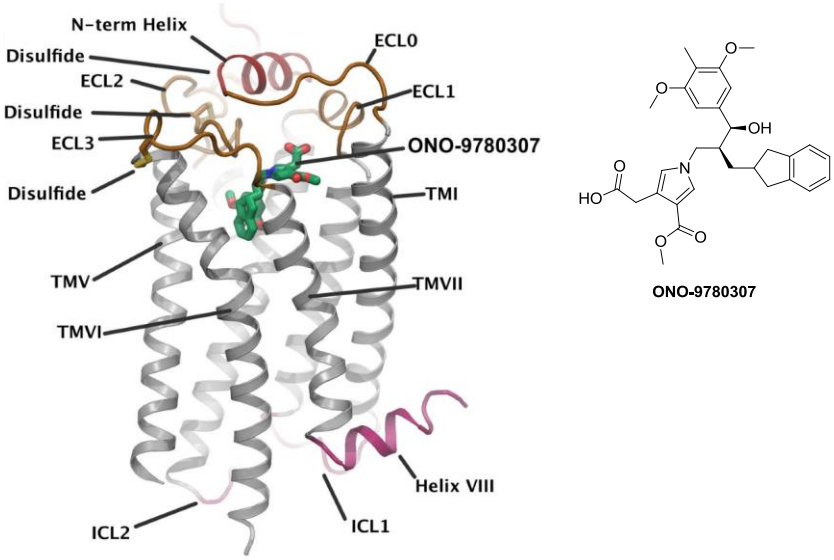
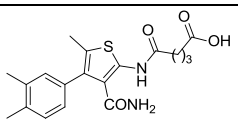
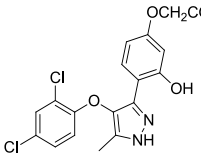
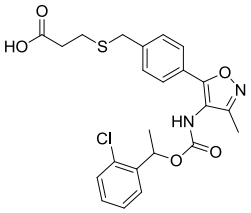


Figure 26. Crystallographic structure of LPA_1 receptor in complex with ONO-9780307. ICL, intracellular loop; ECL, extracellular loop; TM, transmembrane helix (Source: *Cell* **2015**, *161*, 1633-1643).

This VS yielded 18 potential hits, which were purchased and screened for antagonist activity at LPA_1, LPA_2 and LPA_3 receptors given their sequence similarity (51.3% between LPA_1 and LPA_2 receptors, and 47.3% between LPA_1 and LPA_3 receptors).

To experimentally confirm the antagonist activity of the compounds, RH7777 cells stably transfected with LPA_1 receptor or B103 cells stably transfected with LPA_2 or LPA_3 receptors were preloaded with Fluo 4-NW, incubated with the compound under study at a fixed dose of 10 μM, and stimulated with LPA (10 μM). The antagonism was measured as the capacity of the compound to decrease the intensity of LPA response. Under these conditions six compounds were able to antagonize at least the 30% of the LPA stimulation, and they were selected to perform complete dose-response curves to obtain half-maximum inhibitory concentration (IC_{50}) values (data not shown). Among the analyzed compounds, we were able to identify one compound selective for each receptor of interest, **VS6** as LPA_1 receptor antagonist and **VS11** as LPA_2 receptor antagonist. It is remarkable that they showed E_{max} and IC_{50} values close to Ki16425,[69] the most potent but non-selective antagonist of the LPA receptors described so far (Table 11).

Table 11. Antagonist activity of compounds at LPA_{1-3} receptors

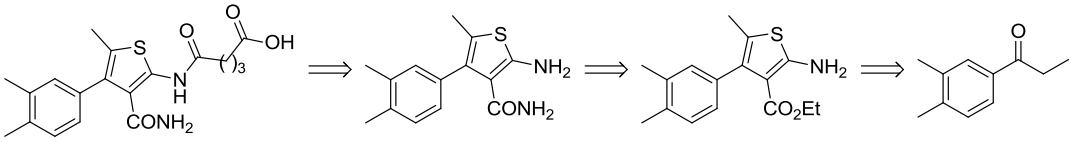
Compound	Structure	IC_{50} (μM)[a] (E_{max} (%)[b])		
		LPA_1	**LPA_2**	**LPA_3**
VS6		10.1 ± 0.9 (50 ± 5)	N. E.[c]	N. E.
VS11		N. E.	1.9 ± 0.6 (88 ± 4)	N. E.
Ki16425		0.8 ± 0.2 (97 ± 4)	1.2 ± 0.6 (92 ± 3)	1.6 ± 0.5 (99 ± 3)

[a]For E_{max} > 30%, IC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate.[b]E_{max} = maximal efficacy of the drug/maximal efficacy of Ki16425, expressed as the percentage.[c]No effect was observed at the highest concentration of compound tested (10 μM).

In order to confirm the structure of the supplied compounds **VS6** and **VS11**, NMR and HPLC-MS analyses were performed. Once their structures were assessed, the next step was to set up synthetic routes that allow to obtain both antagonists, in order to confirm their activity at LPA_1 and LPA_2 receptors with independently synthesized compounds, and to start the optimization process.

2.3.1. Synthesis of antagonist **VS6**

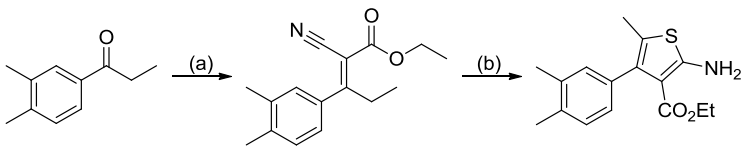
The synthetic route to obtain thiophene **VS6** (Figure 27) could start by Gewald reaction between commercially available 1-(3,4-dimethylphenyl)propan-1-one and ethyl 2-cyanoacetate to build the heterocyclic central core. Next, the transformation of the ester group in primary amide, followed by coupling reaction with glutaric anhydride would afford the desired compound **VS6**.



VS6

Figure 27. Retrosynthetic analysis for compound **VS6**

Thus, 2-aminothiophene **90** was prepared in a two-step Gewald reaction (Scheme 29),[103] in which the Knoevenagel condensation product was reacted with elemental sulfur to afford thiophene **90**.

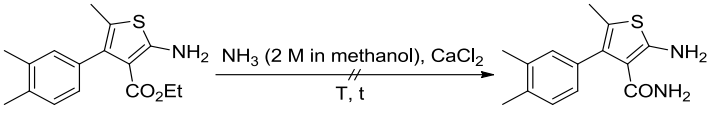


90

Scheme 29. Reagents and conditions: (a) Ethyl 2-cyanoacetate, $TiCl_4$, Et_3N, THF, 0ºC to rt, 12 h; (b) S_8, Et_2NH, THF, rt, 12 h, 32%.

Intermediate **90** was then treated with a methanolic solution of ammonia, using calcium chloride as Lewis acid, to obtain the amide derivative **91**.[104] However, neither termal conditions nor MW radiation yielded the expected amide **91**, recovering only starting material (Table 12).

Table 12. Experimental conditions to obtain amide **91**

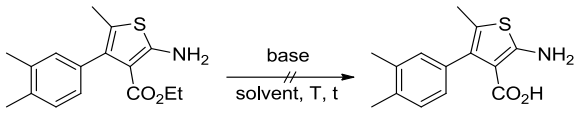


90 **91**

Conditions	T (ºC)	t
Thermal	60	24 h
MW	160	5 min

In view of these results, the possibility of obtaining **91** by hydrolysis of ester **90** followed by transformation into the amide was considered. However, none of the tested reaction conditions (Table 13) afforded the desired product.

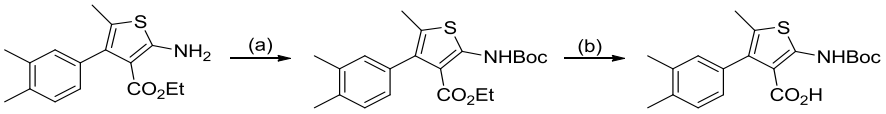
Table 13. Experimental conditions for hydrolysis of ester **90**



90

Conditions	Base	Solvent	T (°C)	t	Results
Thermal	LiOH	THF/H_2O (3:1)	80	24 h	**90**
MW	NaOH	Dioxane	160	4 min	**90**
MW	NaOH	Dioxane	160	7 min	Byproducts

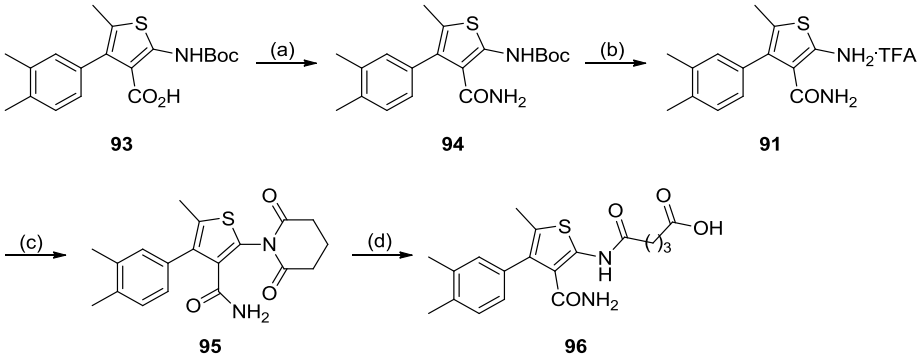
The lack of reactivity of the ester group of compound **90** could be due to the presence of the primary amine in position 2 of the thiophene ring, which may exert an electron-donor effect, making difficult the amidation or the hydrolysis of the ester. For this reason, the amine was protected with a Boc group, previous to its treatment with potassium hydroxide in a mixture of ethanol/water at 60°C for 12 h, conditions that allowed to obtain acid **93** with high yield (Scheme 30).



90 **92** **93**

Scheme 30. Reagents and conditions: (a) Boc_2O, DMAP, THF, rt, 12 h, 28%; (b) KOH, EtOH:H_2O, 60°C, 12 h, 89%.

Next, the formation of the primary amide **94** was accomplished using a methanolic solution of ammonia, in the presence of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) as coupling reagents (Scheme 31). The removal of the Boc group provided 2-aminothiophene **91**, which was treated with glutaric anhydride and subsequently opened in basic media (Scheme 31) to afford final compound **96** (**VS6**).



Scheme 31. Reagents and conditions: (a) 0.5 M NH_3 in dioxane, EDC, HOBt, DIPEA, DCM, rt, 13 h, 70%; (b) TFA, DCM, rt, 12 h, 99%; (c) glutaric anhydride, Et_3N, DMAP, DCM, reflux, 12 h, 33%; (d) Et_3N, H_2O, reflux, 15 min, 72%.

2.3.2. Synthesis of antagonist **VS11**

Obtention of compound **VS11** was planned in four steps (Figure 28), starting with a Friedel-Crafts acylation of resorcinol with 2-(2,4-dichlorophenoxy)acetyl chloride. After attempting different conditions for this reaction (Table 14), only the use of the complex boron trifluoride diethyl etherate at reflux and in DCM, in order to improve the solubility of the materials, afforded the desired compound **97** with a moderate yield (21%) after 24 h of reaction. A reduction of the reaction time with the objective to decrease the formation of byproducts, led to similar results (Table 14).

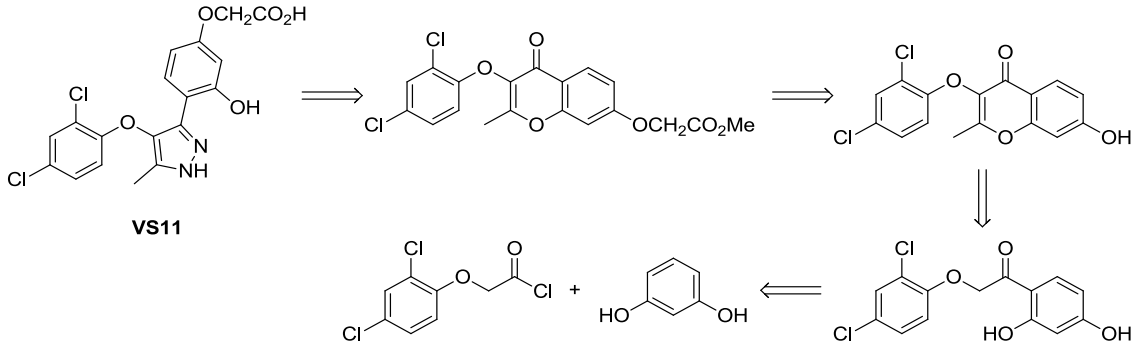
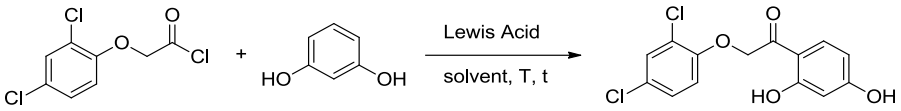


Figure 28. Retrosynthetic analysis for compound **VS11**.

Table 14. Experimental conditions to obtain intermediate **97**

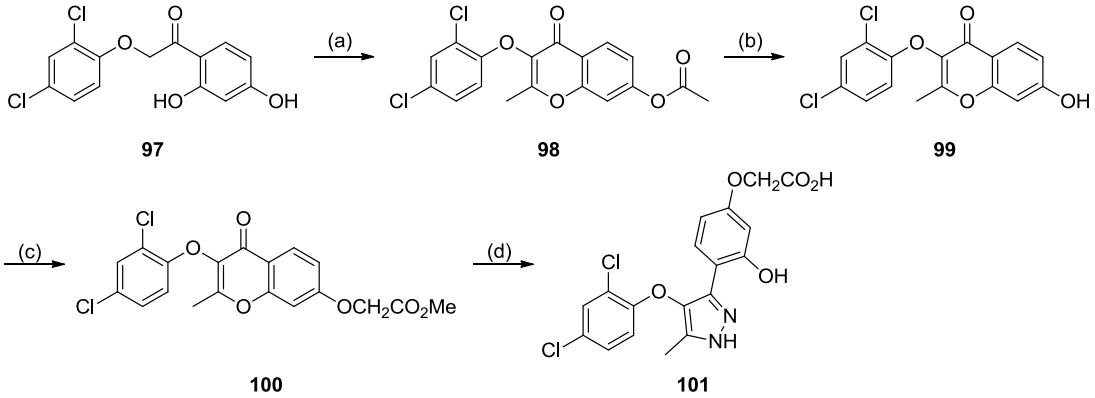


97

Lewis Acid	Solvent	T	t (h)	Result
$AlCl_3$	DCM	Reflux	12	Complex reaction mixture
$AlCl_3$	DCM	r.t.	24	Complex reaction mixture
$BF_3{\cdot}Et_2O$	-	Reflux	24	**97** (<5%) + Byproducts
$BF_3{\cdot}Et_2O$	-	r.t.	24	Starting materials
$BF_3{\cdot}Et_2O$	DCM	Reflux	24	**97** (21%)[a] + Byproducts
$BF_3{\cdot}Et_2O$	DCM	Reflux	4	**97** (20%)[a] + Byproducts

[a] Yield of isolated product **97** after chromatography purification.

Next, Allan−Robinson reaction between compound **97** and acetic anhydride afforded chromone **98** in quantitative yield, which was, after hydrolysis of acetyl group in acid media, alkylated with methyl 2-bromoacetate to yield intermediate **100** with good yield. Finally, opening of the pyrone ring with subsequent cyclization into the corresponding pyrazole and simultaneous hydrolysis of the methyl ester gave desired compound **101** (**VS11**) (Scheme 32).



97 **98** **99**

100 **101**

Scheme 32. Reagents and conditions: (a) Acetic anhydride, Et_3N, NaOAc, 140°C, 2.5 h, 99%; (b) HCl, EtOH, reflux, 2 h, 99%; (c) methyl 2-bromoacetate, K_2CO_3, acetone, reflux, 3 h, 51%; (d) 65% $N_2H_4{\cdot}H_2O$, EtOH, reflux, 30 min, 99%.

2.3.3. Antagonist activity of compounds **96** (**VS6**) and **101** (**VS11**)

Once established the independent synthesis of the antagonists identified in the VS, we determined their antagonist activity at LPA_{1-3} receptors. The obtained results confirmed the IC_{50} values showed by commercially supplied compounds **VS6** and **VS11** (Table 15).

Table 15. Antagonist activity of compound **96**, **101**, **VS6**, **VS11** and **Ki16425** at LPA_{1-3} receptors

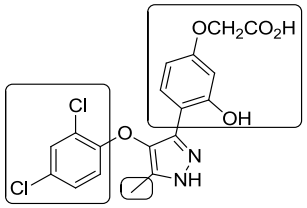
Compound	**IC_{50} (μM)[a] [E_{max} (%)][b]**		
	LPA_1 receptor	**LPA_2 receptor**	**LPA_3 receptor**
96	45 ± 3 [33 ± 2]	N. E.[c]	N. E.
VS6	10.1 ± 0.9 [50 ± 5]	N. E	N. E.
101	N. E.[c]	5.5 ± 0.7 [84 ± 3]	N. E.
VS11	N. E.	1.9 ± 0.6 [88 ± 4]	N. E.
Ki16425	0.8 ± 0.2 [97 ± 4]	1.2 ± 0.6 [92 ± 3]	1.6 ± 0.5 [99 ± 3]

[a]For E_{max} > 30%, IC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [b]E_{max} = maximal efficacy of the drug/maximal efficacy of Ki16425, expressed as the percentage. [c]No effect was observed at the highest concentration of compound tested (10 μM).

Considering the limited knowledge about the role of LPA_2 receptor in SCI, the selective antagonist **101** (**VS11**) has been selected in order to establish which structural characteristics are important for the antagonist activity, and to elucidate the specific involvement of LPA_2 receptor in this pathology.

2.3.4. Structural modifications of compound **101**

We started studying whether the 2,4-dichlorophenoxy system, the 3-hydroxyphenoxyacetic fragment or the methyl group in position 5 of the pyrazole ring are essential for the antagonism (Figure 29).

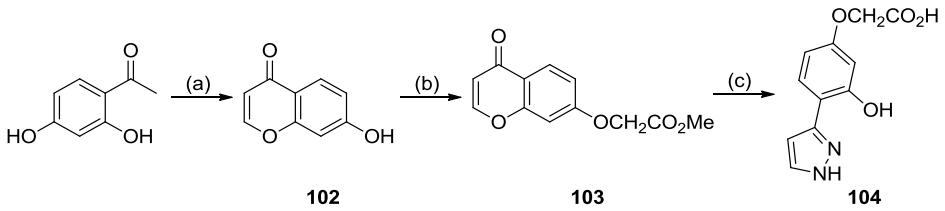


101

Figure 29. Study of the influence of the 2,4-dichlorophenoxy system, the 3-hydroxy phenoxyacetic fragment or the methyl group in the antagonist activity of compound **101**.

2.3.4.1. Modifications in the 2,4-dichlorophenoxy system

Regarding the importance of the 2,4-dichlorophenoxy system, the pyrazole **104** was synthesized. The hydroxychromone **102** was prepared from the commercially available 1-(2,4-dihydroxyphenyl)ethanone by treatment with triethyl orthoformate and perchloric acid. Then, alkylation with methyl bromoacetate and treatment with an excess of hydrazine led to pyrazole **104** (Scheme 33).



102 **103** **104**

Scheme 33. Reagents and conditions: (a) $CH(OEt)_3$, 70% $HClO_4$, H_2O, rt, 13 h, 47%; (b) methyl bromoacetate, K_2CO_3, acetone, reflux, 3 h, 24%; (f) 65% $N_2H_4 \cdot H_2O$, EtOH, reflux, 30 min, 98%.

Biological assay revealed that compound **104** was inactive at LPA_2 receptor, highlighting the importance of 2,4-dichlorophenoxy system for the antagonist activity. Therefore, the effect of the chlorine atoms was studied with the synthesis of compounds **105**-**107** (Figure 30).

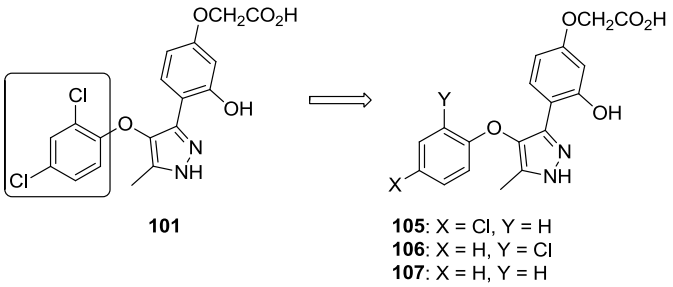
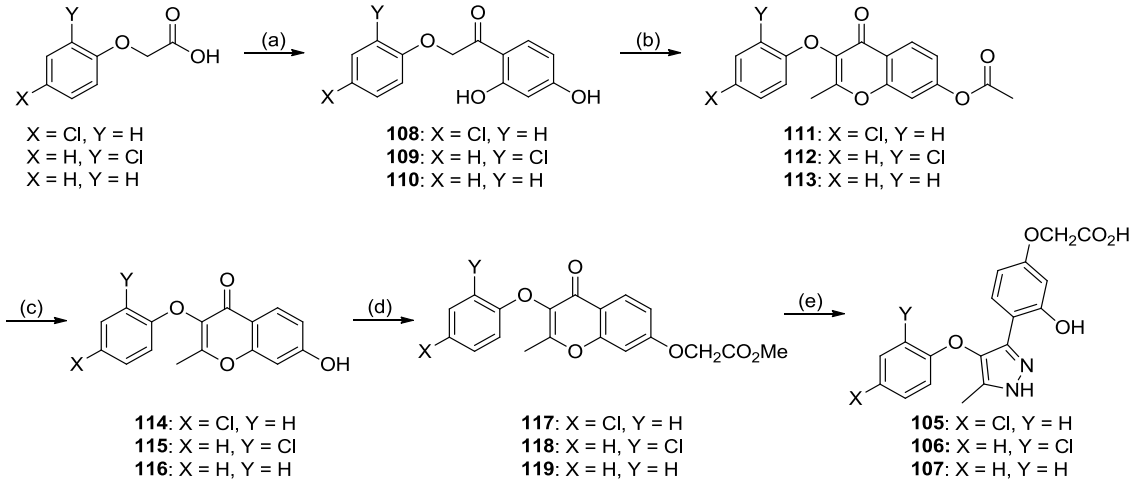


Figure 30. Study of the influence of chlorine atoms in the antagonist activity of compound **101**.

Thus, Friedel-Craft reaction between the corresponding 2-phenoxyacetyl chloride and resorcinol, followed by Allan-Robinson reaction, hydrolysis of acetyl group, alkylation with methyl bromoacetate and, finally, pyrazole ring formation, allowed to obtain compounds **105**-**107** (Scheme 34).



Scheme 34. Reagents and conditions: (a) i) $SOCl_2$, toluene, 110°C, 12 h, 99%; ii) resorcinol, $BF_3{\cdot}Et_2O$, DCM, reflux, 4 h, 10-20%; (b) acetic anhydride, Et_3N, NaOAc, 140°C, 2.5 h, 81-99%; (c) HCl, EtOH, reflux, 2 h, 75-99%; (d) methyl bromoacetate, K_2CO_3, acetone, reflux, 3 h, 50-62%; (f) 65% $N_2H_4{\cdot}H_2O$, EtOH, reflux, 30 min, 99%.

Determination of the antagonist character of compounds **105**-**107** revealed their lack of activity at LPA_2 receptor. Hence, the 2,4-dichlorophenoxy system was kept costant in the next modifications.

2.3.4.2. Modifications in the 3-hydroxyphenoxyacetic fragment

Regarding the variations in the 3-hydroxyphenoxyacetic fragment, first we studied the need of the carboxylic acid chain and the hydroxy group with the synthesis of compound **120** (Figure 31).

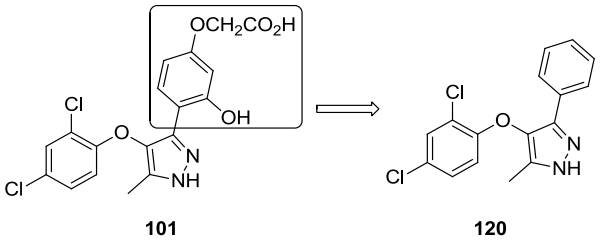
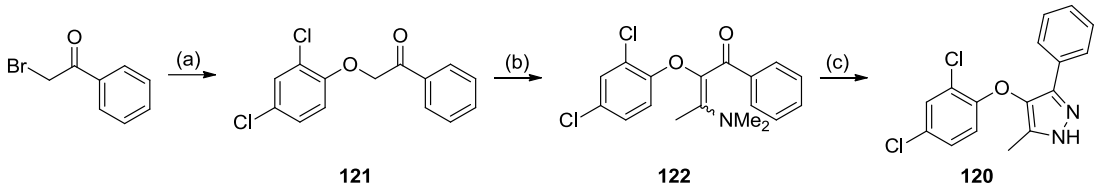


Figure 31. Study of the influence of carboxylic acid chain and hydroxy group in the antagonist activity of compound **101**.

Phenylpyrazole **120** was prepared by Williamson reaction between 2-bromo-1-phenylethanone and 2,4-dichlorophenol, followed by enaminone formation to obtain intermediate **122**, whose treatment with an excess of hydrazine led to the desired target compound (Scheme 35).



Scheme 35. Reagents and conditions: (a) 2,4-Dichlorophenol, DBU, DMF, MW, 140°C, 30 min, 70%; (b) 1,1-dimethoxy-*N*,*N*-dimethylethanamine, 90°C, 4 h, 55%; (c) 65% $N_2H_4 \cdot H_2O$, EtOH, reflux, 1 h, 48%.

The biological assay revealed that compound **120** was inactive, highlighting the importance of the substituents in the 3-hydroxyphenoxyacetic fragment for the antagonist activity. Therefore, we studied the influence of these substituents with the synthesis of compounds **123**-**125** (Figure 32).

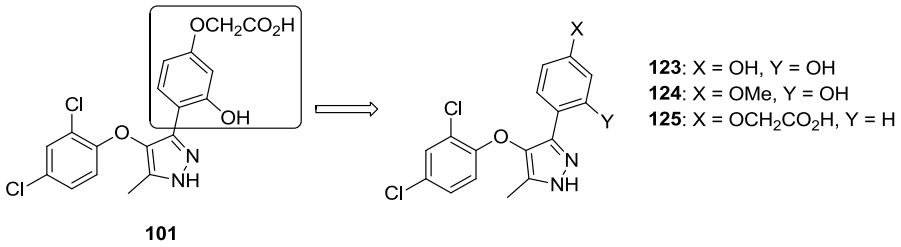
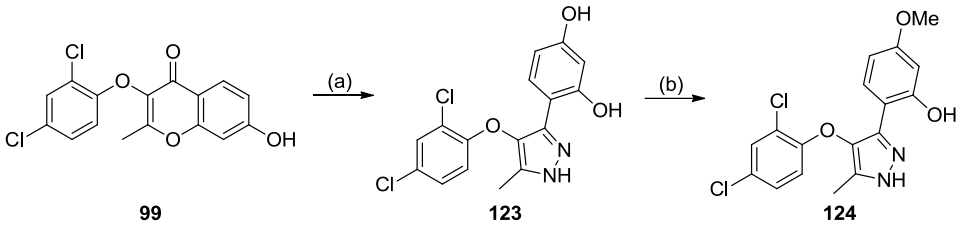


Figure 32. Study of the influence of the substituents in the 3-hydroxyphenoxyacetic fragment for the antagonist activity of compound **101**.

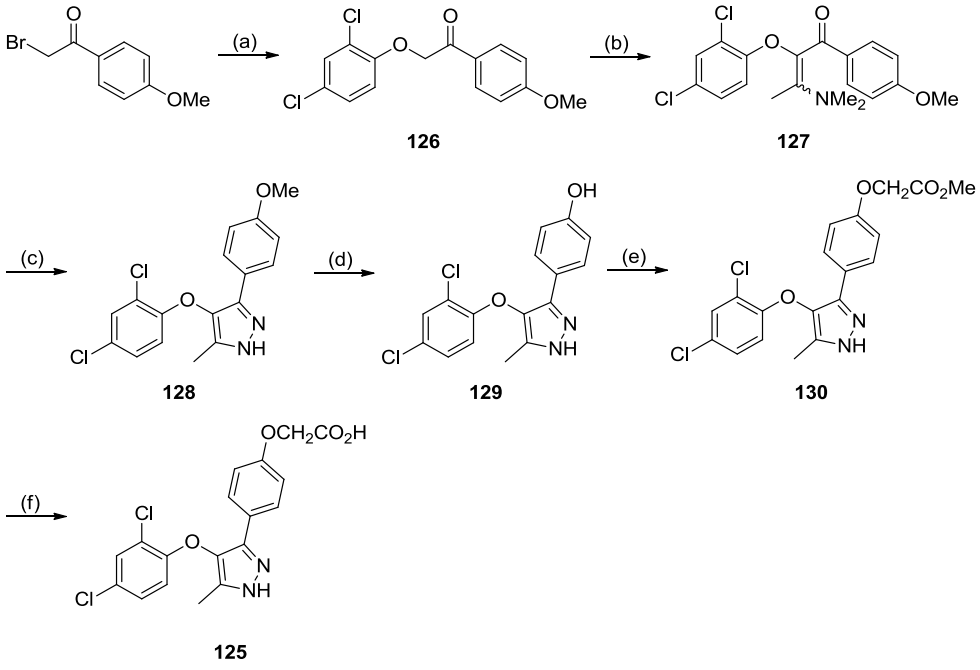
Compounds **123** and **124** were obtained from chromone **99**, which by reaction with an excess of hydrazine, followed by methylation led to desired compounds (Scheme 36).



99 **123** **124**

Scheme 36. Reagents and conditions: (a) 65% $N_2H_4 \cdot H_2O$, EtOH, reflux, 30 min, 65%; (b) CH_3I, K_2CO_3, acetone, 65°C, 6 h, 19%.

In the case of compound **125**, Williamson reaction between 2-bromo-1-(4-methoxyphenyl)ethanone and 2,4-dichlorophenol, using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base under MW irradiation, led to intermediate **126**. Then, treatment with 1,1-dimethoxy-*N*,*N*-dimethylethanamine yielded enaminone **127**, which was reacted with hydrazine and then with BBr_3 to obtain pyrazole **129**. Finally, alkylation with methyl bromoacetate and hydrolysis led to compound **125** (Scheme 37).



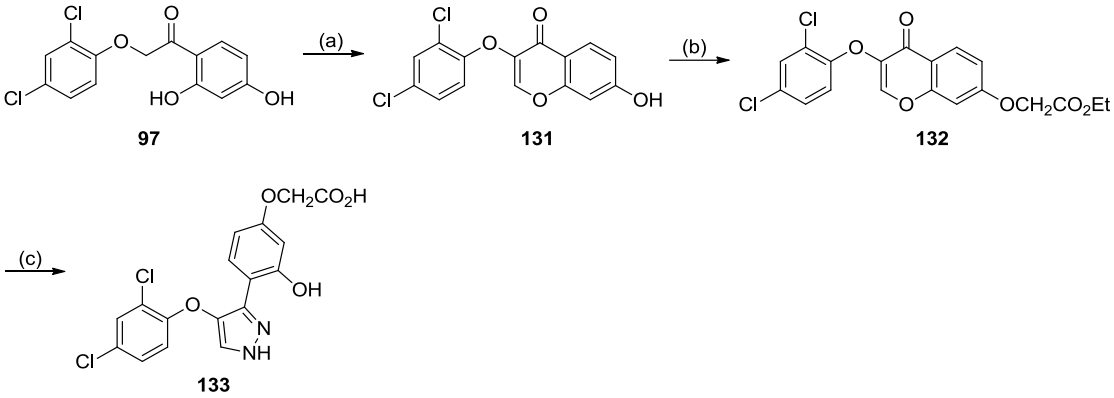
126 **127** **128** **129** **130** **125**

Scheme 37. Reagents and conditions: (a) 2,4-Dichlorophenol, DBU, DMF, MW, 140°C, 30 min, 80%; (b) 1,1-dimethoxy-*N*,*N*-dimethylethanamine, 90°C, 4 h, 52%; (c) 65% $N_2H_4 \cdot H_2O$, EtOH, reflux, 1 h, 44%; (d) BBr_3, DCM, -78°C to rt, 21 h, 70%; (e) methyl bromoacetate, K_2CO_3, DMF, -20°C to rt, 24 h, 32%; (f) 1 M NaOH, 1,4-dioxane, 60°C, 2 h, 99%.

Determination of the antagonist character of compounds **123**-**125** revealed their lack of activity, showing that modifications in the 3-hydroxyphenoxyacetic fragment are detrimental for antagonism at LPA_2 receptor.

2.3.4.3. Modification in pyrazole ring

Up to this moment, the modifications done in the 2,4-dichlorophenoxy system and in the 3-hydroxyphenoxyacetic fragment showed that both fragments are essential for antagonist activity. Hence, compound **133** was prepared in order to study the influence of the methyl group in position 5 of the pyrazole ring. Its synthesis started from derivative **97**, which by reaction with $BF_3{\cdot}Et_2O$ and methanesulfonyl chloride, alkylation with ethyl bromoacetate of the resulting hydroxychromone **131**, and reaction with hydrazine, afforded desired pyrazole **133** (Scheme 38).



Scheme 38. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, CH_3SO_2Cl, DMF, 100°C, 1.5 h, 97%; (b) ethyl bromoacetate, K_2CO_3, acetone, reflux, 3 h, 91%; (f) 65% $N_2H_4{\cdot}H_2O$, EtOH, reflux, 30 min, 58%.

Once compound **133** was synthesized, it was tested for its antagonist activity at LPA_2 receptor, presenting only a weak antagonist character (E_{max} = 35 ± 8%, IC_{50} = 33.6 ± 0.3 µM).

In summary, the results obtained up to this moment suggest that the 2,4-dichlorophenoxy system and the 3-hydroxyphenoxyacetic fragment are essential for the antagonist activity. However, it is important at this point to establish, at least as proof of concept, whether an LPA_2 selective antagonist is able to induce a beneficial effect in SCI. Towards this aim, we have started the first experiments needed to assess the suitability of antagonist **101** for these in vivo studies. In this context, we have determined whether the antagonism observed at LPA_2 receptor is due to a direct binding to the receptor and the ability of the compound to cross biological membranes, important feature for subsequent in vivo administration.

2.3.5. Binding affinity of compound **101** for LPA_2 receptor

Binding affinity was determined with the BSI technique, following a similar procedure to the one employed for compound **(*S*)-3a**, but, in this case, using membrane homogenates containing LPA_2 receptor. The obtained K_D value indicated a high binding affinity of compound **101** for the LPA_2 receptor (K_D = 19.8 nM), comparable to the one obtained for the endogenous ligand LPA (K_D = 6.7 nM) (Figure 33, Table 16).

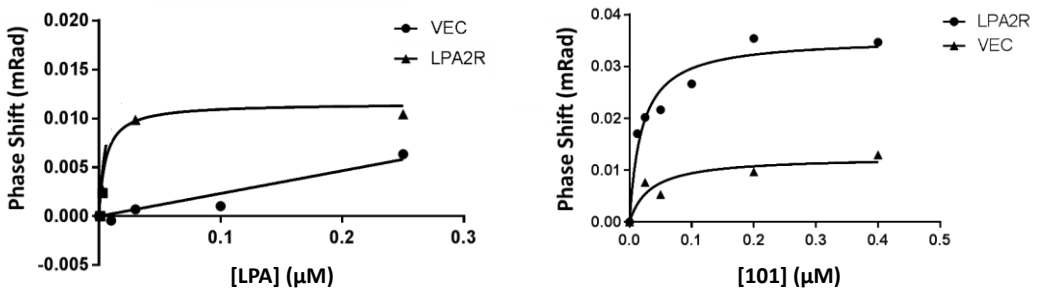


Figure 33. Representative plots of BSI signal versus ligand concentration for the determination of binding constant. Each data point represents the average of at least four independent measurements. Membranes from cells transfected with the empty plasmid -without any receptor (VEC)- were used as negative control.

Table 16. Binding constants determined by BSI at LPA_2 receptor

Compound	K_D (nM)[a]	R^2 (%)[b]
101	19.8	95
LPA	6.7	96

[a] K_D values are the means from two or three independent experiments performed in triplicate. The s.e.m. is in all cases within a 10% of the mean value. [b] A goodness of fit (R^2) greater than 90% is considered adequate.

2.3.6. Cell permeability of compound **101**

Determination of cell permeability is an important aspect of the drug development process and it can be estimated with the parallel artificial membrane permeability assay (PAMPA), which determines the permeability of substances from a donor compartment, through a lipid-infused artificial membrane, into an acceptor compartment. Using this technique, compound **101** showed a moderate permeability value (P) of 1.2×10^{-6} cm/s, considering as reference values $P < 10^{-7}$ cm/s for low permeable compounds and $P > 10^{-5}$ cm/s for highly permeable molecules.

Overall, the obtained results point out that compound **101** can be used in an in vivo model of SCI, as proof of concept of the relevance of the LPA_2 receptor in this

pathology, experiments currently ongoing in collaboration with Prof. Rubèn Lopez Valés, at Universitat Autònoma de Barcelona (Spain). Hence, if these experiments confirm the suitability of LPA_2 receptor antagonists in SCI, a medicinal chemistry program around compound **101** will be carried out in order to obtain most potent antagonists. The synthesis of these second generation compounds will include the replacement of the chlorine atoms with other substituents, the modification of the length of carboxylic chain, and the substitution of pyrazole ring for other heterocycles.

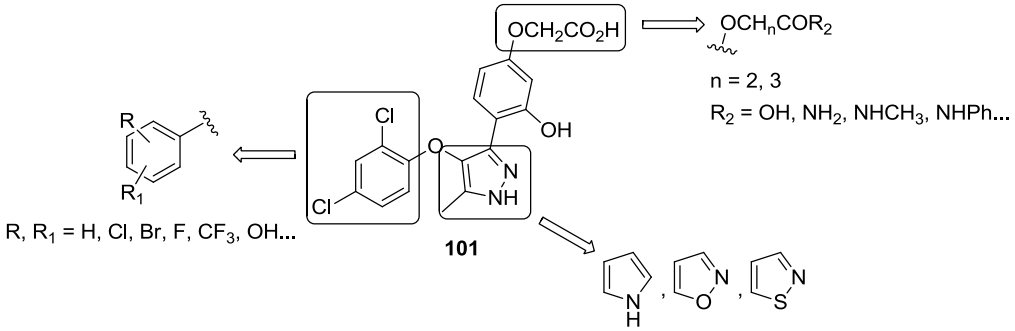


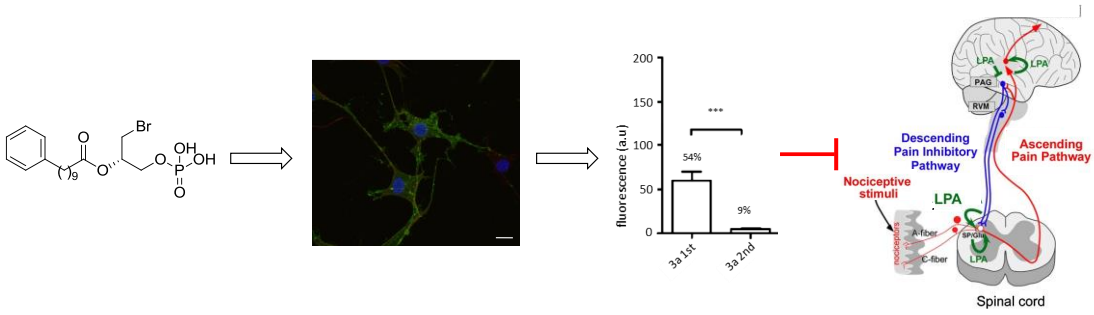
Figure 34. Medicinal chemistry program around compound **101**.

CONCLUSIONS

3. CONCLUSIONS

In the present work we have addressed the development of new potent and selective LPA_1 receptor agonists, as antinociceptive agents, and of new selective antagonists of LPA_1 and LPA_2 receptors, as research tools to elucidate the specific involvement of these receptors in the pathophysiology of SCI.

With respect to the first goal, an exhaustive SAR study of several series of compounds, initially based on the endogenous ligand LPA, was performed and compound **(*S*)-3a** stood out for its high activity (EC_{50} = 0.24 ± 0.09 µM) and affinity (K_D = 19.9 nM) for the LPA_1 receptor, and selectivity towards LPA_2 and LPA_3 receptors. In addition, this derivative promotes the internalization of LPA_1 receptor and its desensitization in sensory neurons, which should lead to the blockade of the signal trasduction involved in pain transmission. Hence, we are currently validating this approach as a possible therapeutic alternative to treat chronic pain in an in vivo model.



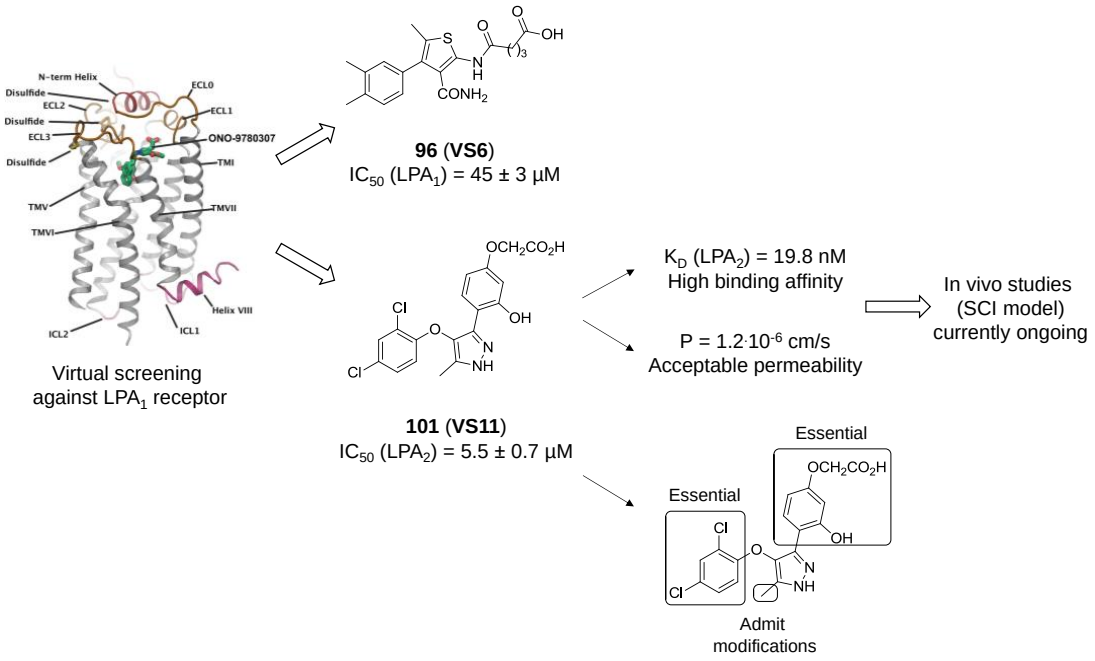
(*S*)-3a
EC_{50} (LPA_1R)= 0.24 ± 0.09 µM

LPA_1 receptor internalization

LPA_1 receptor desensitization in DRG neurons

Blockade of pain transmission

Regarding the second part of this work, a VS has allowed us to identify two potent and selective antagonists of LPA_1 and LPA_2 receptors. Once their synthetic routes were set up and their antagonist activities were confirmed, structural modifications of the LPA_2 receptor antagonist **101** (**VS11**) were carried out to determine which parts of the molecule are essential for its antagonist activity. Then, derivative **101** was selected in order to establish if an LPA_2 selective antagonist is able to induce a beneficial effect in SCI, as proof of concept. This compound shows high binding affinity for LPA_2 receptor (K_D = 19.8 nM) and an acceptable cell permeability value (P = $1.2x10^{-6}$ cm/s), important features for subsequent in vivo studies, which are currently ongoing in our laboratory.



EXPERIMENTAL SECTION

4. EXPERIMENTAL SECTION

4.1. Synthesis

Unless otherwise stated, the starting materials, reagents, and solvents were purchased as high-grade commercial products from Sigma-Aldrich, ABCR, Acros, Biotage, Fluka, Lancaster, Scharlab, or Panreac. Dichloromethane, diethyl ether and tetrahydrofuran (THF) were dried using a Pure Solv^{TM} Micro 100 Liter solvent purification system. Triethylamine and pyridine were dried over CaH_2 and distilled prior to its use. Alcohol-free chloroform was obtained by washing with water, drying over $MgSO_4$, filtration and distillation over P_2O_5. Ethylenediamine was dried over 4Å molecular sieves, distilled and used immediately. All non-aqueous reactions were performed under an argon atmosphere in oven-dried glassware. Reactions under MW irradiation were performed in a Biotage Initiatior 2.5 reactor.

Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254), with detection by UV light (λ = 254 nm), ninhydrin solution, or 10% phosphomolybdic acid solution in ethanol. Flash chromatography was performed on glass column using silica gel type 60 (particle size 230-400 mesh, Merck), or on a Varian 971-FP flash purification system, using silica gel cartridges (Varian, particle size 50 μm).

All compounds were obtained as oils, except for those whose melting points (m.p.) are indicated, which were solids. M.p. were determined on a Stuart Scientific electrothermal apparatus. Infrared (IR) spectra were measured on a Bruker Tensor 27 instrument equipped with a Specac ATR accessory of 5200-650 cm^{-1} transmission range; frequencies (ν) are expressed in cm^{-1}. Optical rotation [α] was measured on a Anton Paar MCP 100 modular circular polarimeter using a sodium lamp (λ = 589 nm) with a 1 dm path length; concentrations are given as g/100 mL. ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra were recorded on a Bruker Avance III 700MHz (^{1}H, 700 MHz; ^{13}C, 175 MHz), Bruker Avance 500MHz (^{1}H, 500 MHz; ^{13}C, 125 MHz; ^{31}P, 202 MHz) or Bruker DPX 300MHz (^{1}H, 300 MHz; ^{13}C, 75 MHz; ^{31}P, 121 MHz) instrument at room temperature at the Universidad Complutense de Madrid's NMR core facility. Proton-

coupled ^{19}F-NMR spectra were recorded on a Bruker DPX 300MHz. Chemical shifts (δ) are expressed in parts per million relative to the residual solvent peak for ^{1}H and ^{13}C nucleus (acetone-*d*$_6$: δ_H = 2.05, δ_C = 29.84; $CDCl_3$: δ_H = 7.26, δ_C = 77.16; DMSO-*d*$_6$: δ_H = 2.50, δ_C = 39.52; methanol-*d*$_4$: δ_H = 3.31, δ_C = 49.00), to internal phosphoric acid for ^{31}P nucleus and to internal (trifluoromethyl)benzene for ^{19}F nucleus; coupling constants (*J*) are in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), qt (quintuplet), m (multiplet), app (apparent) and br (broad). 2D NMR experiments (COSY, HMQC and HMBC) of representative compounds were carried out to assign protons and carbons of the new structures. High resolution mass spectrometry (HRMS) was carried out on a FTMS Bruker APEX Q IV spectrometer in electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) mode at Universidad Complutense de Madrid's mass spectrometry core facility. For all final compounds, purity was determined by HPLC-MS, and satisfactory chromatograms confirmed a purity of at least 95% for all tested compounds. HPLC-MS analysis was performed using an Agilent 1200LC-MSD VL instrument. LC separation was achieved with a Zorbax Eclipse XDB-C18 column (5 µm, 4.6 mm x 150 mm) or Zorbax SB-C3 (5 µm, 2.1 mm x 50 mm) together with a guard column (5 µm, 4.6 mm x 12.5 mm). The gradient mobile phases consisted of A (95:5 water/methanol) and B (5:95 water/methanol) with 0.1% ammonium hydroxide and 0.1% formic acid as the solvent modifiers, and the gradients are indicated in Table 17. MS analysis was performed with an ESI source. The capillary voltage was set to 3.0 kV and the fragmentor voltage was set at 25 eV. The drying gas temperature was 350 ºC, the drying gas flow was 10 L/min, and the nebulizer pressure was 20 psi.

Table 17. HPLC gradients

Method A		**Method B**		**Method C**	
Column SB-C3 Buffers water/methanol		Column XDB-C18 Buffers water/methanol		Column XDB-C18 Buffers water/methanol	
t (min)	**% B**	**t (min)**	**% B**	**t (min)**	**% B**
0	0	0	0	0	0
2	0	2	0	2	0
8	60	10	50	8	60
20	100	20	100	20	100
25	100	50	100	25	100
30	0	60	0	30	0

4.2. Synthesis of new agonists for the LPA_1 receptor

4.2.1. General procedures

4.2.1.1. Hydrogenation of alkenes and deprotection of dibenzyl phosphates and benzyl ethers

The corresponding alkene or benzylated derivative was dissolved in absolute ethanol (0.2 mL/mg) and the solution was pumped through a H-Cube® continuous-flow hydrogenation reactor using a 10% Pd/C CatCart® cartridge, under full-H_2 mode at a flow-rate of 1 mL/min at room temperature (for alkenes and dibenzyl phosphates) or 60°C (for benzyl ethers). Solvent was then removed under reduced pressure to afford the corresponding compound in quantitative yield, which was used without further purification.

4.2.1.2. Mesylation of alcohols

To a cooled (0°C) stirred solution of the corresponding alcohol (1 equiv) and triethylamine (3 equiv) in anhydrous dichloromethane (3.5 mL/mmol), methanesulfonyl chloride (1.5 equiv) was added dropwise. The reaction mixture was stirred at 0°C for 10 min and then at room temperature for 1 h. Afterward, the mixture was partitioned between ethyl acetate and brine. The organic layer was separated, washed with a saturated aqueous solution of $NaHCO_3$ and with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield the corresponding mesylate, which was used in the next step without further purification.

4.2.1.3. Tosylation of alcohols

To a cooled (0°C) stirred solution of the corresponding alcohol (1 equiv) and pyridine (3 equiv) in anhydrous dichloromethane (1.2 mL/mmol), *p*-toluenesulfonyl chloride (1.5 equiv) was added portionwise. The reaction mixture was stirred at 0°C for 10 min and then at room temperature for 16 h. Afterward, the mixture was washed with water and with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to afford the corresponding tosylated derivative.

4.2.1.4. Di-*tert*-butyl malonate alkylation

Di-*tert*-butyl malonate (1.5 equiv) was added dropwise to a stirred suspension of NaH (1.5 equiv, 60% dispersion in oil) in a 2:1 mixture of anhydrous *N,N*-dimethylformamide (DMF)/THF (6 mL/mmol) at 0ºC, and the mixture was stirred at room temperature for 15 min. A solution of the corresponding mesylate (1 equiv) in anhydrous THF (3 mL/mmol) was added, followed by sodium iodide (1.1 equiv), and the resulting mixture was heated at 80ºC overnight. Afterward, the reaction was cooled to room temperature and quenched by addition of a saturated aqueous solution of NH_4Cl. The mixture was then diluted with water, and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography to afford the corresponding alkylated product.

4.2.1.5. α-Fluorination of malonate derivatives

The corresponding malonate derivative (1 equiv) was added to a suspension of NaH (2 equiv, 60% dispersion in oil) in anhydrous THF (4 mL/mmol) at 0ºC, and the reaction mixture was warmed up to room temperature and then stirred at 70°C for 12 h. The solution was cooled to room temperature and diluted with anhydrous THF (8 mL/mmol) and DMF (8 mL/mmol). Afterward, Selectfluor® was added (2 equiv) at 0ºC and the solution was stirred at this temperature for 4 h and at room temperature overnight. The reaction mixture was quenched by addition of water and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to afford the corresponding fluorinated derivative.

4.2.1.6. Deprotection of acetals using polystyrene-supported *p*TsOH

The corresponding acetal (1 equiv) was dissolved in methanol (3 mL/g resin) and polystyrene-supported *p*TsOH (PS-*p*TsOH) was added (0.3 equiv, 4.56 mmol *p*TsOH/g resin). The reaction was stirred at room temperature overnight. Afterward, the mixture was filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography to afford the corresponding diol.

4.2.1.7. Regioselective esterification of oleoyl chloride with diols

To a stirred solution of the corresponding diol (1.5 equiv) in anhydrous dichloromethane (12 mL/mmol) at -78°C, 2,4,6-collidine (2 equiv) and oleoyl chloride (1 equiv) were added. The mixture was stirred for 24 h while gradually warming to room temperature. After this time, solvent was evaporated under reduced pressure and the residue was treated with ethyl acetate, removing the 2,4,6-collidine hydrochloride by filtration. The filtrate was concentrated and the crude was purified by flash chromatography to yield the corresponding ester.

4.2.1.8. Deprotection of *tert*-butyl esters and alkyl di-*tert*-butyl phosphates

TFA (25 or 75 equiv) was added to a solution of the corresponding *tert*-butyl derivative (1 equiv) in anhydrous dichloromethane (20 mL/mmol) and the reaction was stirred at room temperature until disappearance of the starting material. The mixture was then treated with brine and the aqueous phase was extracted with dichloromethane. The combined organic layers were washed with water and brine, dried over Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to afford the corresponding phosphate or carboxylic acid.

4.2.1.9. Methylation of alcohols with trimethylsilyldiazomethane

To a vigorously stirred mixture of the corresponding alcohol (1 equiv) and tetrafluoroboric acid (1 equiv, 35% aqueous solution) in anhydrous dichloromethane (4 mL/mmol) at 0ºC, trimethylsilyldiazomethane (1 equiv, 2 M in diethyl ether) was added dropwise, waiting for the yellow colour to disappear before each addition. The stirring was continued at 0ºC and three further portions of trimethylsilyldiazomethane (0.5 equiv, 0.25 equiv and 0.25 equiv) were added dropwise at intervals of 20 min. Afterward, the mixture was stirred at 0ºC for further 30 min, poured into water and extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to afford the pure compound.

4.2.1.10. Sonogashira coupling reaction of alkynes with methyl-8-bromooctanoate

1,3-Bis(1-adamantyl)imidazolium chloride (0.15 equiv), CuI (0.225 equiv), $[(\pi\text{-allyl})PdCl]_2$ (0.075 equiv), and Cs_2CO_3 (1.4 equiv) were added in turn to a thoroughly dried vial. A 2:1 mixture of anhydrous diethyl ether and DMF (1.5 mL/mmol ester) was added, followed by the corresponding alkyne (1.3 equiv) and methyl-8-bromooctanoate (1 equiv). The vial was sealed with a Teflon-lined cap and the heterogeneous reaction mixture was stirred vigorously at 55ºC for 16 h. The solvents were then evaporated under reduced pressure and the residue was purified by flash chromatography to yield the desired coupled product.

4.2.1.11. Stereoselective *cis*-hydrogenation of alkynes with P-2 Ni catalyst

$Ni(OAc)_2 \cdot 4H_2O$ (0.2 equiv) was suspended in absolute ethanol (3 mL/mmol alkyne) at room temperature. $NaBH_4$ (0.2 equiv) was added and the mixture was stirred for 15 min. Then, argon atmosphere was replaced by hydrogen (balloon). Freshly distilled ethylenediamine was added (1.5 equiv) and the reaction was stirred for 15 min. A solution of the corresponding alkyne (1 equiv) in absolute ethanol (3 mL/mmol) was then added, and the reaction was stirred under hydrogen atmosphere at room temperature for 2 h. After this time, the reaction mixture was filtered through a pad of Celite, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to afford the corresponding *Z*-alkene, which was used in the next step without further purification.

4.2.1.12. Hydrolysis of methyl esters

The corresponding methyl ester (1 equiv) was dissolved in THF (10 mL/mmol) and a solution of lithium hydroxide (2 equiv) in water (1.5 mL/mmol) was added. The reaction was stirred at room temperature overnight. Then, the mixture was acidified with a 20% aqueous solution of HCl and extracted with dichloromethane. The organic phase was dried over Na_2SO_4, filtered, and concentrated, affording the corresponding carboxylic acid in quantitative yield.

4.2.1.13. Phosphorylation of alcohols using phosphoramidites

To a solution of the corresponding alcohol (1 equiv) in anhydrous dichloromethane (12 mL/mmol), 1*H*-tetrazole (3 equiv, 0.45 M in acetonitrile) and the corresponding phosphoramidite (2 equiv) were added at room temperature and the reaction mixture was stirred until total consumption of the alcohol. Then it was cooled to -30ºC, *m*CPBA (2 equiv) was added and the mixture was stirred at -30ºC for 90 min. The reaction was quenched by addition of a 10% aqueous solution of $Na_2S_2O_3$ (12 mL/mmol alcohol) and allowed to warm to room temperature. Then, the mixture was extracted with dichloromethane and the extracts were successively washed with a 20% aqueous solution of K_2CO_3 and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to afford the corresponding pure phosphate derivative.

4.2.1.14. Regioselective esterification of carboxylic acids with diols

To a solution of the corresponding carboxylic acid (1 equiv), DCC (1.1 equiv) and DMAP (0.2 equiv) in anhydrous dichloromethane (10 mL/mmol acid) at -20ºC, a solution of the corresponding diol (2 equiv) in anhydrous dichloromethane (2 mL/mmol) was added and the reaction was stirred overnight while warming up to room temperature. Afterward, the mixture was concentrated under reduced pressure, and

the residue was dissolved in carbon tetrachloride and filtered to remove dicyclohexylurea. The filtrate was evaporated and purified by flash chromatography to afford the corresponding pure ester.

4.2.1.15. Wittig reaction of ω-bromoacids and aromatic aldehydes

A mixture of the corresponding ω-bromoacid (1.2 equiv) and triphenylphosphine (6 equiv) in anhydrous toluene (2.4 mL/mmol) was refluxed for 24 h. Then, the mixture was allowed to cool to room temperature, the solvent was evaporated and the residue was washed with hexane to remove excess of triphenylphosphine, and dried. The obtained phosphonium salt (1.2 equiv) was dissolved in anhydrous THF (4.2 mL/mmol), and lithium hexamethyldisilazane (2.7 equiv, 1 M in toluene) was added dropwise at -20ºC, turning the solution orange. The mixture was stirred for 30 min followed by addition of the corresponding aldehyde (1 equiv) at -20ºC and the stirring was continued overnight at room temperature. The reaction mixture was acidified with 1 M HCl and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography to yield the corresponding alkene.

4.2.1.16. Ring-opening reaction of epoxides with TBABr

To a solution of the corresponding oxirane (1 equiv) and TBABr (3 equiv) in freshly distilled alcohol-free chloroform (10 mL/mmol), TFA (1.5 equiv) was added and the reaction was stirred for 10 min at room temperature. Then, the reaction mixture was passed through a silica gel column (5 g/mmol) prepared in chloroform and it was washed with the same solvent (100 mL/mmol). The solvent was evaporated under reduced pressure to give the corresponding bromoalcohol, which was used in the next step without further purification.

4.2.1.17. Esterification of carboxylic acids with bromoalcohols and primary alcohols

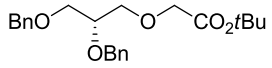
To a stirred solution of the corresponding carboxylic acid (1 equiv), DCC (1.1 equiv) and DMAP (0.2 equiv) in anhydrous dichloromethane (10 mL/mmol), a solution of the corresponding alcohol (1 equiv) in anhydrous dichloromethane (2 mL/mmol) was added at room temperature and the reaction was stirred until consumption of the starting material. Afterward, the mixture was concentrated under reduced pressure, and the residue was dissolved in carbon tetrachloride and filtered to remove dicyclohexylurea. The filtrate was concentrated and it was purified by flash chromatography to yield the corresponding ester.

4.2.2. Synthesis of final compounds **1a**-**f**

4.2.2.1. Synthesis of diol **2**

tert-Butyl {[(2*R*)-2,3-bis(benzyloxy)propyl]oxy}acetate, 1

(*S*)-(-)-2,3-Bis(benzyloxy)propan-1-ol (0.56 mL, 2.20 mmol, 1 equiv) was added dropwise to a stirred suspension of NaH (176 mg, 4.40 mmol, 2 equiv, 60% dispersion in oil) in anhydrous THF (10 mL) at 0°C. After stirring the mixture at room temperature for 30 min, *tert*-butyl bromoacetate (0.49 mL, 3.31 mmol, 1.5 equiv) and TBAI (41 mg, 0.11 mmol, 0.05 equiv) were added, and the resulting mixture was heated at 50°C overnight. After cooling to room temperature, the reaction was quenched by addition of water and concentrated under reduced pressure. The residue was dissolved with dichloromethane, washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate, 9:1 to 8:2) to afford pure compound **1** in 22% yield.

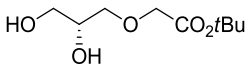


1

R_f: 0.52 (hexane/ethyl acetate, 8:2)
IR (ATR): 1738 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.36 (s, 9H, $3CH_3$); 3.47-3.65 (m, 4H, $2CH_2$); 3.72 (qt, *J* = 4.7, 1H, CH); 3.88 (s, 2H, CH_2CO_2tBu); 4.44 (s, 2H, $PhCH_2$); 4.61 (s, 2H, $PhCH_2$); 7.11-7.29 (m, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 28.1 ($3CH_3$); 69.4, 70.4, 71.7 ($3CH_2$); 72.3, 73.4 ($2PhCH_2$); 77.4 (CH); 81.5 (C); 127.5, 127.6 ($2CH_{Ar}$); 127.7 ($2CH_{Ar}$); 127.8 ($2CH_{Ar}$); 128.3 ($2CH_{Ar}$); 128.4 ($2CH_{Ar}$); 138.4, 138.8 ($2C_{Ar}$); 169.6 (CO)
MS (ESI, *m/z*): 387.7 $[M+H]^+$

tert-Butyl {[(2*R*)-2,3-dihydroxypropyl]oxy}acetate, 2

Following the general procedure 4.2.1.1., diol **2** was obtained from **1** (190 mg, 0.49 mmol) at 60ºC in quantitative yield.



2

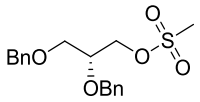
R_f: 0.11 (hexane/ethyl acetate, 8:2)
IR (ATR): 3376 (O-H); 1738 (C=O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.49 (s, 9H, $3CH_3$); 3.50-3.64 (m, 4H, $2CH_2$); 3.78 (qt, *J* = 5.3, 1H, CH); 4.87 (s, 2H, CH_2CO_2tBu)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 28.1 ($3CH_3$); 63.7 (CH_2OH); 68.8 ($\underline{C}H_2CO_2tBu$); 70.5 (CH); 73.4 (CH_2O); 82.5 (C); 170.6 (CO)
MS (ESI, *m/z*): 229.1 $[M+Na]^+$

4.2.2.2. Synthesis of diols **5** and **7**

(2*R*)-2,3-Bis(benzyloxy)propyl methanesulfonate, 3

Following the general procedure 4.2.1.2., mesylate **3** was obtained from (*S*)-(-)-2,3-bis(benzyloxy)propan-1-ol (0.35 mL, 1.39 mmol) in 80% yield.

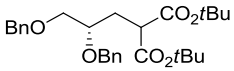


3

R_f: 0.51 (hexane/ethyl acetate, 7:3)
IR (ATR): 1354 (SO_2)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.95 (s, 3H, CH_3); 3.60 (dd, *J* = 4.8, 1.5, 2H, $C\underline{H}_2OBn$); 3.86 (m, 1H, CH); 4.31 (dd, *J* = 11.0, 5.6, 1H, ½$C\underline{H}_2OMs$); 4.43 (dd, *J* = 11.0, 3.7, 1H, ½$C\underline{H}_2OMs$); 4.54 (s, 2H, $PhC\underline{H}_2$); 4.66 (s, 2H, $PhC\underline{H}_2$); 7.27-7.39 (m, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 37.5 (CH_3); 68.5, 69.4 ($2CH_2$); 72.5, 73.6 ($2Ph\underline{C}H_2$); 75.7 (CH); 127.8 ($2CH_{Ar}$); 127.9 ($2CH_{Ar}$); 127.91 ($2CH_{Ar}$); 128.0 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 137.7 ($2C_{Ar}$)
MS (ESI, *m/z*): 368.2 $[M+NH_4]^+$

Di-*tert*-butyl [(2*S*)-2,3-bis(benzyloxy)propyl]propanedioate, 4

Following the general procedure 4.2.1.4., compound **4** was obtained from mesylate **3** (391 mg, 1.12 mmol) in 76% yield. Chromatography: hexane to hexane/ethyl acetate, 9:1.



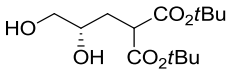
4

R_f: 0.65 (hexane/ethyl acetate, 9:1)
IR (ATR): 1727 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.42 (s, 9H, $3CH_3$); 1.45 (s, 9H, $3CH_3$); 2.04-2.10 (m, 2H, $C\underline{H}_2CH(CO_2tBu)_2$); 3.46 (dd, *J* = 8.9, 6.0, 1H, $C\underline{H}(CO_2tBu)_2$); 3.55 (dd, *J* = 4.8, 0.8, 2H, $C\underline{H}_2OBn$); 3.62-3.68 (m, 1H, $C\underline{H}OBn$); 4.53 (d, *J* = 11.5, 1H, ½$PhC\underline{H}_2$); 4.54 (s, 2H, $PhC\underline{H}_2$); 4.69 (d, *J* = 11.5, 1H, ½$PhC\underline{H}_2$); 7.31-7.38 (m, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.9 ($3CH_3$); 28.0 ($3CH_3$); 31.3 ($\underline{C}H_2CH(CO_2tBu)_2$); 50.4 ($\underline{C}H(CO_2tBu)_2$); 72.4, 72.8 ($2Ph\underline{C}H_2$); 73.4 ($\underline{C}H_2OBn$); 76.0 ($\underline{C}HOBn$); 81.4 (2C); 127.55, 127.58 ($2CH_{Ar}$); 127.6 ($2CH_{Ar}$); 127.9 ($2CH_{Ar}$); 128.3 ($2CH_{Ar}$); 128.4 ($2CH_{Ar}$); 138.6 ($2C_{Ar}$); 169.0 (2CO)

MS (ESI, *m/z*): 471.2 $[M+H]^+$

Di-*tert*-butyl [(2*S*)-2,3-dihydroxypropyl]propanedioate, 5

Following the general procedure 4.2.1.1., diol **5** was obtained from **4** (327 mg, 0.74 mmol) at 60ºC in quantitative yield.

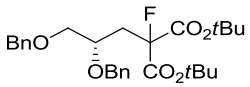


5

R_f: 0.37 (hexane/ethyl acetate, 8:2)
IR (ATR): 3408 (O-H); 1726 (C=O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.46 (s, 9H, $3CH_3$); 1.47 (s, 9H, $3CH_3$); 1.76 (ddd, *J* = 14.8, 9.6, 5.3, 1H, ½CH_2CH(CO_2tBu)$_2$); 2.02 (ddd, *J* = 14.8, 9.5, 3.3, 1H, ½CH_2CH(CO_2tBu)$_2$); 3.39-3.48 (m, 3H, CH_2OH, CH(CO_2tBu)$_2$); 3.52-3.63 (m, 1H, CHOH)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 28.2 ($3CH_3$); 28.23 ($3CH_3$); 33.7 (CH_2CH(CO_2tBu)$_2$); 52.0 (CH(CO_2tBu)$_2$); 67.4 (CH_2OH); 70.9 (CHOH); 82.7 (2C); 170.4, 170.8 (2CO)
MS (ESI, *m/z*): 289.1 $[M-H]^-$

Di-*tert*-butyl [(2*S*)-2,3-bis(benzyloxy)propyl](fluoro)propanedioate, 6

Following the general procedure 4.2.1.5., compound **6** was obtained from **4** (400 mg, 0.85 mmol) in quantitative yield. Chromatography: hexane to hexane/ethyl acetate, 8:2.

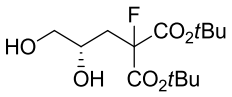


6

R_f: 0.53 (hexane/ethyl acetate, 8:2)
IR (ATR): 1736 (C=O); 1148 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.35 (s, 9H, $3CH_3$); 1.45 (s, 9H, $3CH_3$); 2.24-2.38 (m, 2H, CH_2CF); 3.53 (d, *J* = 4.8, 2H, CH_2OBn); 3.85-3.93 (m, 1H, CH); 4.51 (d, *J* = 11.3, 1H, ½PhCH_2); 4.51 (s, 2H, PhCH_2); 4.60 (d, *J* = 11.3, 1H, ½PhCH_2); 7.28-7.33 (m, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.7 ($3CH_3$); 27.8 ($3CH_3$); 36.6 (CH_2CF); 72.6, 73.0 (2PhCH_2); 73.3 (CH_2OBn); 74.7 (CH); 82.4, 82.8 (2C(CH_3)$_3$); 127.5 ($2CH_{Ar}$); 127.6 ($2CH_{Ar}$); 128.1 ($2CH_{Ar}$); 128.32 ($2CH_{Ar}$); 128.33 ($2CH_{Ar}$); 138.35, 138.7 ($2C_{Ar}$); 169.4, 170.1 (2CO); CF not observed
MS (ESI, *m/z*): 506.3 $[M+NH_4]^+$

Di-*tert*-butyl [(2*S*)-2,3-dihydroxypropyl](fluoro)propanedioate, 7

Following the general procedure 4.2.1.1., diol **7** was obtained from **6** (211 mg, 0.43 mmol) at 60ºC in quantitative yield.



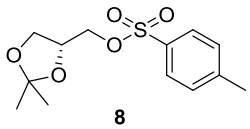
7

R_f: 0.14 (hexane/ethyl acetate, 8:2)
IR (ATR): 3407 (O-H); 1743 (C=O); 1154 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.51 (s, 18H, $6CH_3$); 2.18 (dt, *J* = 15.3, 3.0, 1H, ½CH_2CF); 2.34 (ddd, *J* = 15.3, 9.2, 9.0, 1H, ½CH_2CF); 2.91-3.20 (br s, 2H, 2OH); 3.50 (dd, *J* = 11.3, 6.8, 1H, ½CH_2OH); 3.65 (dd, *J* = 11.3, 3.3, 1H, ½CH_2OH); 3.96-4.06 (m, 1H, CH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.7 ($3CH_3$); 27.75 ($3CH_3$); 37.2 (d, *J* = 20.8, CH_2CF); 66.5 (CH_2OH); 67.3 (d, *J* = 2.8, CH); 83.9 (2C(CH_3)$_3$); 93.4 (d, *J* = 196.0, CF); 165.3 (d, *J* = 25.0, CO); 165.8 (d, *J* = 26.1, CO)

4.2.2.3. Synthesis of diol **10**

[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]methyl 4-methylbenzenesulfonate, 8

Following the general procedure 4.2.1.3., tosylate **8** was obtained from (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (0.5 mL, 4.05 mmol) in 86% yield. Chromatography: hexane to hexane/ethyl acetate, 1:1.

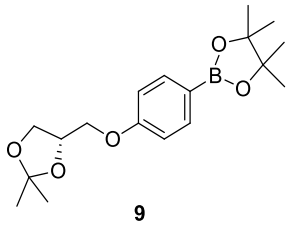


8

R_f: 0.28 (hexane/ethyl acetate, 5:1)
IR (ATR): 1364 (SO_2); 1179 (SO_2)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.31 (s, 3H, CH_3); 1.34 (s, 3H, CH_3); 2.45 (s, 3H, CH_3C_{Ar}); 3.77 (dd, *J* = 8.8, 5.1, 1H, ½CH_2O); 3.94-4.06 (m, 3H, CH_2OS, ½CH_2O); 4.24-4.31 (m, 1H, CH); 7.35 (d, *J* = 8.1, 2H, $2CH_{Ar}$); 7.80 (d, *J* = 8.3, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.8 (CH_3C_{Ar}); 25.3, 26.8 ($2CH_3$); 66.4 (CH_2O); 69.6 (CH_2OS); 73.1 (CH); 110.2 (C); 128.2 ($2CH_{Ar}$); 130.1 ($2CH_{Ar}$); 132.8, 145.2 ($2C_{Ar}$)
$[\alpha]_D^{20}$: -4.2 (c = 1.41, methanol)
MS (ESI, *m/z*): 287.1 $[M+H]^+$

2-(4-{[(4*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]methoxy}phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 9

To a solution of tosylate **8** (343 mg, 1.2 mmol, 1.2 equiv) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (220 mg, 1.0 mmol, 1 equiv) in anhydrous DMF (5 mL), Cs_2CO_3 (652 mg, 2.0 mmol, 2 equiv) was added. The mixture was heated at 90ºC for 16 h. Afterward, the mixture was partitioned between ethyl acetate and water and the aqueous layer was extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane to hexane/ethyl acetate, 7:3) to afford pure compound **9** in 84% yield.

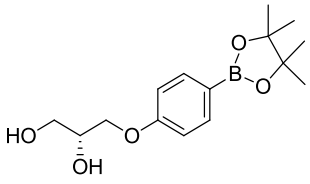


9

R_f: 0.63 (hexane/ethyl acetate, 8:2)
IR (ATR): 1362 (B-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.33 (s, 12H, $4CH_{3\ boronate}$); 1.40 (s, 3H, $CH_{3\ acetal}$); 1.46 (s, 3H, $CH_{3\ acetal}$); 3.90 (dd, *J* = 8.5, 5.8, 1H, ½CH_2O); 3.96 (dd, *J* = 9.6, 6.0, 1H, ½CH_2O); 4.08 (dd, *J* = 9.5, 5.4, 1H, ½CH_2O); 4.17 (dd, *J* = 8.5, 6.4, 1H, ½CH_2O); 4.48 (qt, *J* = 5.9, 1H, CH); 6.90 (d, *J* = 8.7, 2H, $2CH_{Ar}$); 7.74 (d, *J* = 8.6, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.0 ($4CH_{3\ boronate}$); 25.5, 26.9 ($2CH_{3\ acetal}$); 67.0, 68.7 ($2CH_2$); 74.1 (CH); 83.7 ($2C_{boronate}$); 109.9 (C_{acetal}); 114.0 ($2CH_{Ar}$); 136.7 ($2CH_{Ar}$); 161.2 (C_{Ar}); CB not observed
$[\alpha]_D^{20}$: +9.2 (c = 0.6, $CHCl_3$)
MS (ESI, *m/z*): 335.2 $[M+H]^+$

(2*R*)-3-[4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy]propane-1,2-diol, 10

Following the general procedure 4.2.1.6., diol **10** was obtained from **9** (425 mg, 1.27 mmol) in 88% yield. Chromatography: hexane/ethyl acetate, 1:1 to 2:8.



10

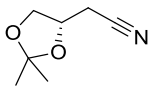
R_f: 0.05 (hexane/ethyl acetate, 7:3)
IR (ATR): 3372 (O-H); 1361 (B-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.33 (s, 12H, $4CH_3$); 2.76 (br s, 2H, OH); 3.72 (dd, *J* = 11.5, 5.5, 1H, ½CH_2OAr); 3.82 (dd, *J* = 11.5, 3.7, 1H, ½CH_2OAr); 4.02-4.04 (m, 2H, CH_2OH); 4.06-4.13 (m, 1H, CH); 6.88 (d, *J* = 8.7, 2H, $2CH_{Ar}$); 7.74 (d, *J* = 8.7, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.0 ($4CH_3$); 63.7 (CH_2OH); 69.0 (CH_2OAr); 70.5 (CH); 83.8 (2C); 114.0 ($2CH_{Ar}$); 136.7 ($2CH_{Ar}$); 161.1 (C_{Ar}); CB not observed
$[\alpha]_D^{20}$: +14.2 (c = 1.70, methanol)
MS (ESI, *m/z*): 295.1 $[M+H]^+$; 312.2 $[M+NH_4]^+$

4.2.2.4. Synthesis of diol **12**

[(4*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]acetonitrile, 11

To a solution of (*S*)-(+)-1,2-isopropylideneglycerol (0.37 mL, 3.0 mmol, 1 equiv) in anhydrous dichloromethane (13.6 mL), pyridine (0.98 mL, 12.1 mmol, 4 equiv) and triflic anhydride (1.0 mL, 6.1 mmol, 2 equiv) were added at -20ºC. The reaction mixture was stirred at this temperature for 30 min. Afterward, the mixture was poured into cold 1M HCl (5 mL) and extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield the corresponding triflate derivative, which was used in the next step without further purification.

To a solution of triflate derivative (793 mg, 3.0 mmol, 1 equiv) in a 7.5:1 mixture of acetonitrile:water (2.8 mL), KCN (234 mg, 3.6 mmol, 1.2 equiv) was added, and the mixture was stirred at room temperature overnight. Then it was concentrated under reduced pressure and the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford nitrile derivative **11** in quantitative yield, without needing further purification.

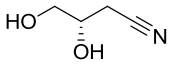


11

R_f: 0.44 (hexane/ethyl acetate, 8:2)
IR (ATR): 2251 (CN); 1070 (C-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.34 (s, 3H, CH_3); 1.45 (s, 3H, CH_3); 2.54-2.68 (dd, *J* = 5.7, 3.1, 2H, CH_2CN); 3.78 (dd, *J* = 8.7, 5.5, 1H, ½CH_2O); 4.14 (dd, *J* = 8.7, 6.0, 1H, ½CH_2O); 4.33 (qt, *J* = 5.7, 1H, CH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 22.8 (CH_2CN); 25.4, 26.9 ($2CH_3$); 68.3 (CH_2O); 71.1 (CH); 110.6 (C); 116.7 (CN)
$[\alpha]_D^{20}$: +0.55 (c = 0.84, methanol)

(3*S*)-3,4-Dihydroxybutanenitrile, 12

Nitrile **11** (207 mg, 1.47 mmol, 1 equiv) was treated with a 9:1 mixture of TFA:MeOH (6.6 mL) at room temperature for 90 min. Afterward, the reaction was concentrated under reduced pressure and the residue was purified by flash chromatography (hexane to hexane/ethyl acetate, 6:4) to afford pure 12 in 61% yield.



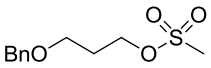
12

R_f: 0.47 (hexane/ethyl acetate, 1:4)
IR (ATR): 3354 (O-H); 2250 (CN); 1026 (C-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.63 (ABX system, *J* = 16.9, 6.8, 4.7, 2H, CH_2CN); 3.53 (ABX system, *J* = 11.2, 5.9, 5.3, 2H, CH_2OH); 3.87 (qt, *J* = 5.7, 1H, CH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.8 (CH_2CN); 64.7 (CH_2OH); 68.1 (CH); 118.2 (CN)
$[\alpha]_D^{20}$: +0.22 (c = 1.56, methanol)

4.2.2.5. Synthesis of alcohol **22**

3-(Benzyloxy)propyl methanesulfonate, 19

Following the general procedure 4.1.1.2., mesylate **19** was obtained from benzyloxypropan-1-ol (0.19 mL, 1.20 mmol) in quantitative yield.

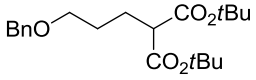


19

R_f: 0.4 (hexane/ethyl acetate, 5:1)
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 2.04 (qt, *J* = 6.1, 2H, CH_2CH_2OMs); 2.96 (s, 3H, CH_3); 3.59 (t, *J* = 5.9, 2H, CH_2OBn); 4.36 (t, *J* = 6.2, 2H, CH_2OMs); 4.51 (s, 2H, $PhCH_2$); 7.27-7.39 (m, 5H, $5CH_{Ar}$)
^{13}C-NMR (DMSO-d_6, 75 MHz): δ 29.6 (CH_2CH_2OMs); 37.2 (CH_3); 65.5 (CH_2OMs); 67.4 (CH_2OBn); 73.2 ($PhCH_2$); 127.7 ($2CH_{Ar}$); 127.8 (CH_{Ar}); 128.5 ($2CH_{Ar}$); 138.1 (C_{Ar})
MS (ESI, *m/z*): 245.1 $[M+H]^+$; 267.0 $[M+Na]^+$

Di-*tert*-butyl [3-(benzyloxy)propyl]propanedioate, 20

Following the general procedure 4.2.1.4., compound **20** was obtained from mesylate **19** (270 mg, 1.11 mmol) in 66% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.

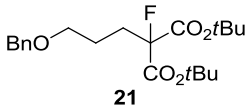


20

R_f: 0.49 (hexane/ethyl acetate, 8:2)
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.45 (s, 18H, 6CH_3); 1.60-1.70 (m, 2H, C$H_2$$CH_2$CH); 1.86-1.94 (m, 2H, C$H_2$CH); 3.14 (t, *J* = 7.5, 1H, CH); 3.49 (t, *J* = 6.4, 2H, CH_2OBn); 4.50 (s, 2H, PhCH_2); 7.24-7.37 (m, 5H, 5CH_{Ar})
^{13}C-NMR (DMSO-d_6, 75 MHz): δ 25.4 (CH_2CH); 27.4 (C$H_2$$CH_2$CH); 28.0 (6$CH_3$); 53.7 (CH); 69.8 (C$H_2$OBn); 72.9 (PhC$H_2$); 81.3 (2C); 127.5 ($CH_{Ar}$); 127.6 (2$CH_{Ar}$); 128.4 (2$CH_{Ar}$); 138.5 ($C_{Ar}$); 168.9 (2CO)
MS (ESI, *m/z*): 365.3 $[M+H]^+$; 387.1 $[M+Na]^+$

Di-*tert*-butyl [3-(benzyloxy)propyl](fluoro)propanedioate, 21

Following the general procedure 4.1.1.5., compound **21** was obtained from **20** (250 mg, 0.71 mmol) in quantitative yield, which was used in next step without further purification.

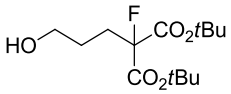


21

R_f: 0.82 (hexane/ethyl acetate, 8:2)
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.49 (s, 18H, 6CH_3); 1.59-1.77 (m, 2H, C$H_2$$CH_2$CF); 2.00-2.24 (m, 2H, CH_2CF); 3.46-3.52 (m, 2H, CH_2OBn); 4.49 (s, 2H, PhCH_2); 7.28-7.37 (m, 5H, 5CH_{Ar})
^{13}C-NMR (DMSO-d_6, 75 MHz): δ 23.6 (d, *J* = 2.9, C$H_2$$CH_2$CF); 28.2 (6$CH_3$); 31.2 (d, *J* = 21.8, CH_2CF); 70.0 (CH_2OBn); 73.1 (PhCH_2); 83.8 (2C(CH_3)$_3$); 100.9 (d, *J* = 165.6, CF); 127.9 (CH_{Ar}); 128.0 (2CH_{Ar}); 128.8 (2CH_{Ar}); 138.9 (C_{Ar}); 165.7 (d, *J* = 25.7, 2CO)
MS (ESI, *m/z*): 400.2 $[M+NH_4]^+$; 405.2 $[M+Na]^+$

Di-*tert*-butyl fluoro(3-hydroxypropyl)propanedioate, 22

Following the general procedure 4.2.1.1., alcohol **22** was obtained from **21** (329 mg, 0.86 mmol) at 60ºC in quantitative yield.



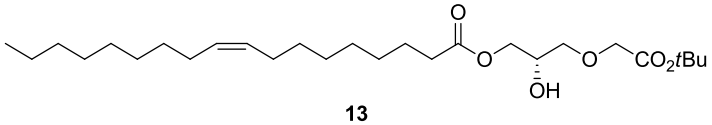
22

R_f: 0.31 (hexane/ethyl acetate, 4:1)
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.50 (s, 18H, 6CH_3); 1.54-1.71 (m, 2H, C$H_2$$CH_2$CF); 1.91-2.20 (m, 2H, CH_2CF); 3.45-3.64 (m, 2H, CH_2OH)
^{13}C-NMR (DMSO-d_6, 75 MHz): δ 26.1 (d, *J* = 2.5, C$H_2$$CH_2$CF); 27.8 (6$CH_3$); 30.2 (d, *J* = 21.8, CH_2CF); 62.1 (CH_2OH); 83.5 (2C(CH_3)$_3$); 96.0 (d, *J* = 196.0, CF); 165.6 (d, *J* = 25.8, 2CO)
MS (ESI, *m/z*): 293.1 $[M+H]^+$

4.2.2.6. Synthesis of esters **13**-**15**, **17**-**18** and **23**

(2*S*)-3-(2-*tert*-Butoxy-2-oxoethoxy)-2-hydroxypropyl (9*Z*)-octadec-9-enoate, 13

Following the general procedure 4.2.1.7., ester **13** was obtained from diol **2** (82 mg, 0.49 mmol) in 36% yield. Chromatography: dichloromethane/ethyl acetate, 100:1 to 5:1.

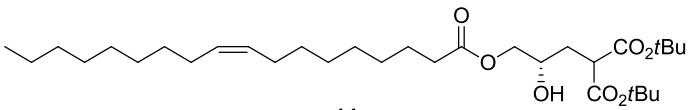


13

R_f: 0.52 (dichloromethane/ethyl acetate, 8:2)
IR (ATR): 3460 (O-H); 1739 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.23-1.36 (m, 20H, $10CH_2$); 1.48 (s, 9H, $3CH_3$); 1.57-1.69 (m, 2H, CH_2CH_2CO); 1.95-2.06 (m, 4H, $2CH_2CH_{alkene}$); 2.34 (t, *J* = 7.6, 2H, CH_2CO); 3.56 (dd, *J* = 10.0, 6.4, 1H, ½$CHCH_2O$); 3.66 (dd, *J* = 10.0, 3.6, 1H, ½$CHCH_2O$); 3.95-4.07 (m, 3H, CH_2CO_2tBu, CH); 4.11-4.21 (m, 2H, CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 28.3 ($3CH_3$); 29.3 ($2CH_2$); 29.32 (CH_2); 29.5 ($2CH_2$); 29.7, 29.8, 29.9, 32.1 ($4CH_2$); 34.3 (CH_2CO); 65.2 (CO_2CH_2); 69.0 (CH); 69.2 (CH_2CO_2tBu); 73.4 ($CHCH_2O$); 82.4 (C); 129.9, 130.2 ($2CH_{alkene}$); 170.4 (CO_2tBu); 174.0 (CO)
MS (ESI, *m/z*): 493.1 $[M+Na]^+$

Di-*tert*-butyl {(2*S*)-2-hydroxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl}propanedioate, 14

Following the general procedure 4.2.1.7., ester **14** was obtained from diol **5** (190 mg, 0.72 mmol) in 99% yield. Chromatography: dichloromethane/ethyl acetate, 100:1 to 5:1.



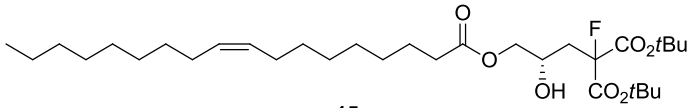
14

R_f: 0.60 (dichloromethane/ethyl acetate, 9:1)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.18-1.37 (m, 20H, $10CH_2$); 1.45 (s, 9H, $3CH_3$); 1.46 (s, 9H, $3CH_3$); 1.56-1.69 (m, 3H, CH_2CH_2CO, ½$CH_2CH(CO_2tBu)_2$); 1.89-2.08 (m, 5H, $2CH_2CH_{alkene}$, ½$CH_2CH(CO_2tBu)_2$); 2.34 (t, *J* = 7.5, 2H, CH_2CO); 3.44 (dd, *J* = 8.1, 6.2, 1H, $CH(CO_2tBu)_2$); 3.84-3.95 (m, 1H, CHOH); 4.01 (dd, *J* = 11.3, 6.7, 1H, ½CO_2CH_2); 4.12 (dd, *J* = 11.3, 3.7, 1H, ½CO_2CH_2); 5.31-5.36 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 28.0 ($3CH_3$); 28.1 ($3CH_3$); 29.2 ($2CH_2$); 29.3, 29.5, 29.7 ($3CH_2$); 29.8 ($2CH_2$); 29.9,

32.1, 32.4 ($3CH_2$); 34.3 ($\underline{C}H_2CO$); 50.9 ($\underline{C}H(CO_2tBu)_2$); 68.2 (CHOH); 68.3 ($CO_2\underline{C}H_2$); 82.0 (2C); 129.9, 130.2 ($2CH_{alkene}$); 168.9, 169.2 ($2\underline{C}O_2tBu$); 174.0 (CO)
MS (ESI, *m/z*): 555.2 $[M+H]^+$

Di-*tert*-butyl fluoro{(2*S*)-2-hydroxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl} propanedioate, 15

Following the general procedure 4.2.1.7., ester **15** was obtained from diol **7** (116 mg, 0.38 mmol) in 60% yield. Chromatography: dichloromethane to dichloromethane/ethyl acetate, 9:1.

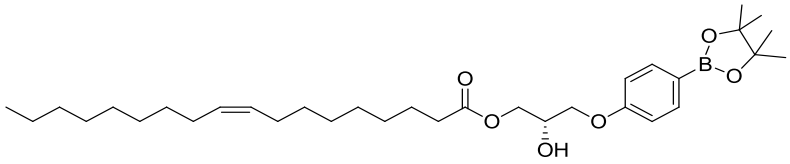


15

R_f: 0.56 (hexane/ethyl acetate, 8:2)
IR (ATR): 3513 (O-H); 1743 (C=O); 1164 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.6, 3H, CH_3); 1.22-1.37 (m, 20H, $10CH_2$); 1.50 (s, 18H, $6CH_3$); 1.60-1.65 (m, 2H, $C\underline{H}_2CH_2CO$); 1.97-2.03 (m, 4H, $2C\underline{H}_2CH_{alkene}$); 2.23-2.44 (m, 4H, $C\underline{H}_2CO$, $C\underline{H}_2CF$); 3.99-4.21 (m, 3H, CO_2CH_2, CH); 5.32-5.36 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.1 (CH_3); 22.7, 24.9 ($2CH_2$); 27.2, 27.24 ($2\underline{C}H_2CH_{alkene}$); 27.7 ($3CH_3$); 27.78 ($3CH_3$); 29.1, 29.2 ($2CH_2$); 29.3 ($2CH_2$); 29.33, 29.5, 29.7, 29.8, 31.9 ($5CH_2$); 34.1 ($\underline{C}H_2CO$); 37.4 (d, *J* = 20.4, CH_2CF); 65.3 (CH); 67.9 ($CO_2\underline{C}H_2$); 83.8, 83.9 ($2\underline{C}(CH_3)_3$); 129.8, 130.0 ($2CH_{alkene}$); 165.5 (d, *J* = 24.8, $\underline{C}O_2tBu$); 166.0 (d, *J* = 25.7, $\underline{C}O_2tBu$); 174.4 (CO); CF not observed
MS (ESI, *m/z*): 590.5 $[M+NH_4]^+$

(2*S*)-2-Hydroxy-3-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy] propyl (9*Z*)-octadec-9-enoate, 16

Following the general procedure 4.2.1.7., ester **16** was obtained from diol **10** (130 mg, 0.44 mmol) in 40% yield. Chromatography: dichloromethane to dichloromethane/ethanol, 100:1.



16

R_f: 0.46 (dichloromethane/ethanol, 20:1)
IR (ATR): 3459 (O-H); 1737 (C=O); 1362 (B-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.26-1.33 (m, 32H, $10CH_2$, $4CH_{3\ boronate}$); 1.56-1.68 (m, 2H, $C\underline{H}_2CH_2CO$); 1.97-2.01 (m, 4H, $2C\underline{H}_2CH_{alkene}$); 2.35 (t,

J = 7.5, 2H, CH_2CO); 2.57 (br s, 1H, OH); 4.00-4.09 (m, 2H, $C\underline{H}_2OAr$); 4.22-4.32 (m, 3H, CH, CO_2CH_2); 5.28-5.40 (m, 2H, $2CH_{alkene}$); 6.90 (d, *J* = 8.7, 2H, $2CH_{Ar}$); 7.75 (d, *J* = 8.6, 2H, $2CH_{Ar}$)

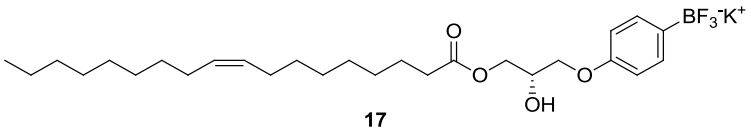
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8 (CH_2); 25.0 ($4CH_3$); 25.04 (CH_2); 27.3, 27.4 ($2\underline{C}H_2CH_{alkene}$); 29.2 ($2CH_2$); 29.3 ($CH_2$); 29.5 ($2CH_2$); 29.7, 29.8, 29.9, 32.0 ($4CH_2$); 34.3 ($\underline{C}H_2CO$); 65.3 ($CO_2\underline{C}H_2$); 68.6 ($\underline{C}H_2OAr$); 68.8 (CH); 83.8 (2C); 114.0 ($2CH_{Ar}$); 129.9, 130.2 ($2CH_{alkene}$); 136.7 ($2CH_{Ar}$); 161.0 (C_{Ar}); 174.1 (CO); CB not observed

$[\alpha]_D^{20}$: +1.5 (c = 1, $CHCl_3$)

MS (ESI, *m/z*): 557.8 $[M-H]^-$

Potassium [4-({(2*S*)-2-hydroxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl}oxy)phenyl] trifluoroborate, 17

To a solution of boronate ester **16** (50 mg, 0.09 mmol, 1 equiv) in methanol (2 mL), potassium hydrogen fluoride was added (111 µL, 0.50 mmol, 5.6 equiv, 4.5 M in water) and the reaction was stirred at room temperature for 30 min. Then, solvent was evaporated under reduced pressure and the crude was dissolved in hot acetone and filtrated. The filtrate was concentrated under reduced pressure to afford intermediate **17** as a syrup in quantitative yield.



17

IR (ATR): 3500 (O-H); 1737 (C=O); 1237 (B-F); 1176 (B-C)

^{1}H-NMR (methanol-d_4, 300 MHz): δ 0.90 (t, *J* = 6.6, 3H, CH_3); 1.30-1.37 (m, 20H, $10CH_2$); 1.60-1.64 (m, 2H, $C\underline{H}_2CH_2CO$); 2.02-2.04 (m, 4H, $2C\underline{H}_2CH_{alkene}$); 2.36 (t, *J* = 7.4, 2H, CH_2CO); 3.94-4.02 (m, 2H, $C\underline{H}_2OAr$); 4.09-4.28 (m, 3H, CH, CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$); 6.79 (d, *J* = 8.0, 2H, $2CH_{Ar}$); 7.42 (d, *J* = 8.3, 2H, $2CH_{Ar}$)

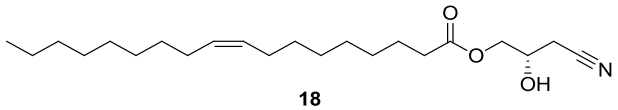
^{13}C-NMR (methanol-d_4, 75 MHz): δ 14.5 (CH_3); 23.7, 26.0 ($2CH_2$); 28.1 ($2\underline{C}H_2CH_{alkene}$); 30.2 ($2CH_2$); 30.3, 30.34, 30.5, 30.6, 30.8, 30.84, 33.1 ($7CH_2$); 34.9 ($\underline{C}H_2CO$); 66.5 ($CO_2\underline{C}H_2$); 69.2 (CH); 69.9 ($\underline{C}H_2OAr$); 114.1 ($2CH_{Ar}$); 130.8, 130.9 ($2CH_{alkene}$); 133.6 ($2CH_{Ar}$); 158.8 (C_{Ar}); 175.4 (CO); CB not observed

$[\alpha]_D^{20}$: +2.4 (c = 0.9, methanol)

MS (ESI, *m/z*): 521.3 $[M-OH]^-$

(2*S*)-3-Cyano-2-hydroxypropyl (9*Z*)-octadec-9-enoate, 18

Following the general procedure 4.2.1.7., ester **18** was obtained from diol **12** (61 mg, 0.60 mmol) in 88% yield. Chromatography: hexane to hexane/ethyl acetate, 6:4.

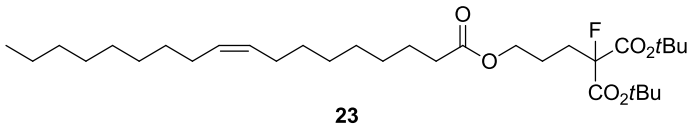


18

R_f: 0.27 (hexane/ethyl acetate, 8:2)
IR (ATR): 3380 (O-H); 2250 (CN); 1740 (C=O); 1115 (C-O); 1093 (C-O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 0.90 (t, J = 6.6, 3H, CH_3); 1.29-1.33 (m, 20H, $10CH_2$); 1.57-1.65 (m, 2H, CH_2CH_2CO); 2.00-2.06 (m, 4H, $2CH_2CH_{alkene}$); 2.36 (t, J = 7.6, 2H, CH_2CO); 2.57-2.74 (m, 2H, CH_2CN); 4.04-4.14 (m, 3H, CH, CO_2CH_2); 5.29-5.39 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 13.5 (CH_3); 20.9 (CH_2CN); 22.8, 24.9 ($2CH_2$); 27.2 ($2CH_2CH_{alkene}$); 29.22, 29.24, 29.3, 29.4, 29.5, 29.7, 29.8, 29.9, 32.1 ($9CH_2$); 33.8 (CH_2CO); 65.3 (CH); 66.5 (CO_2CH_2); 118.7 (CN); 129.8, 129.9 ($2CH_{Alkene}$); 173.9 (CO)
$[\alpha]_D^{20}$: +0.53 (c = 1.00, methanol)
MS (ESI, *m/z*): 383.4 $[M+NH_4]^+$; 388.3 $[M+Na]^+$

Di-*tert*-butyl fluoro{3-[(9*Z*)-octadec-9-enoyloxy]propyl}propanedioate, 23

Following the general procedure 4.2.1.7., ester **23** was obtained from alcohol **22** (199 mg, 0.68 mmol) in 70% yield. Chromatography: dichloromethane.



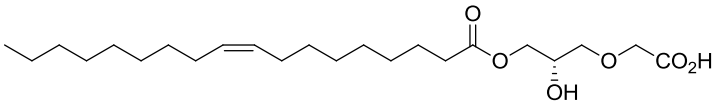
23

R_f: 0.69 (hexane/ethyl acetate, 1:1)
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, J = 6.8, 3H, CH_3); 1.23-1.37 (m, 20H, $10CH_2$); 1.46 (s, 3H, CH_3); 1.50 (s, 15H, $5CH_3$); 1.56-1.64 (m, 2H, CH_2CH_2CO); 1.69-1.79 (m, 2H, CH_2CH_2CF); 1.97-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.07-2.20 (m, 2H, CH_2CF); 2.29 (t, J = 7.6, 2H, CH_2CO); 4.08 (t, J = 6.3, 2H, CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (DMSO-d_6, 75 MHz): δ 14.5 (CH_3); 22.7 (d, J = 3.2, CH_2CH_2CF); 23.1, 25.3 ($2CH_2$); 27.6, 27.61 ($2CH_2CH_{alkene}$); 28.2 ($6CH_3$); 28.3, 29.5, 29.54, 29.6, 29.7, 29.9, 30.1, 30.2 ($8CH_2$); 30.9 (d, J = 21.9, CH_2CF); 32.3 (CH_2); 34.7 (CH_2CO); 63.9 (CO_2CH_2); 84.0 ($2C(CH_3)_3$); 94.8 (d, J = 196.5, CF); 130.2, 130.4 ($2CH_{alkene}$); 165.7 (d, J = 25.4, $2CO_2tBu$); 174.2 (CO)
MS (ESI, *m/z*): 557.5 $[M+H]^+$

4.2.2.7. Synthesis of final compounds **1a**-**f**

({(2*S*)-2-Hydroxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl}oxy)acetic acid, 1a

Following the general procedure 4.2.1.8., compound **1a** was obtained from *tert*-butyl ester **13** (48 mg, 84 µmol) and TFA (0.16 mL, 2.10 mmol) in 52% yield. Chromatography: ethyl acetate/ethanol, 20:1 to 1:5.

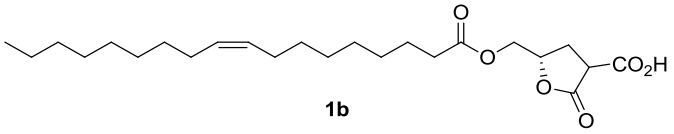


1a

R_f: 0.11 (ethyl acetate/ethanol, 8:2)
IR (ATR): 3301 (O-H); 1737 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.6, 3H, CH_3); 1.26-1.29 (m, 20H, $10CH_2$); 1.52-1.65 (m, 2H, CH_2CH_2CO); 1.93-2.05 (m, 4H, $2CH_2CH_{alkene}$); 2.30 (t, *J* = 7.5, 2H, CH_2CO); 3.32-3.60 (m, 2H, $CHCH_2O$); 3.76-3.92 (br s, 2H, CH_2CO_2H); 3.95-4.19 (m, 3H, CO_2CH_2, CH); 5.26-5.35 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.9, 25.0 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.3, 29.4, 29.47, 29.48, 29.7, 29.8, 29.9, 32.1 ($9CH_2$); 34.2 (CH_2CO); 64.8 (CO_2CH_2); 65.8 (CH); 70.5 (CH_2CO_2H); 72.1 ($CHCH_2O$); 130.0, 130.2 ($2CH_{alkene}$); 173.4, 173.8 (2CO)
$[\alpha]_D^{20}$: -1.6 (c = 1.00, $CHCl_3$)
HRMS (ESI, *m/z*): calculated for $C_{23}H_{41}O_6$ ($[M-H]^-$): 413.2909, found: 413.2890

(5*S*)-5-{[(9*Z*)-Octadec-9-enoyloxy]methyl}-2-oxotetrahydrofuran-3-carboxylic acid, 1b

Following the general procedure 4.2.1.8., compound **1b** was obtained from di-*tert*-butyl ester **14** (169 mg, 0.30 mmol) and TFA (0.58 mL, 7.53 mmol) in 88% yield. Chromatography: ethyl acetate/ethanol, 20:1 to 1:5.



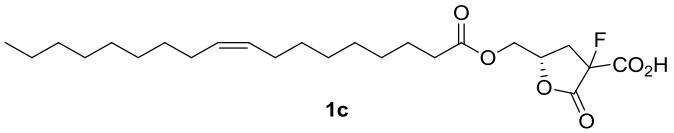
1b

R_f: 0.13 (ethyl acetate/ethanol, 8:2)
^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture of diastereoisomers A:B (1:1): δ 0.88 (t, *J* = 7.2, 3H, CH_3); 1.27-1.30 (m, 20H, $10CH_2$); 1.61-1.64 (m, 2H, CH_2CH_2CO); 1.97-2.03 (m, 4H, $2CH_2CH_{alkene}$); 2.35 (dt, *J* = 7.7, 1.8, 2H, CH_2CO); 2.37-2.41 (m, 1H, $½CH_2CHCO_2H_A$); 2.47-2.54 (m, 1H, $½CH_2CHCO_2H_B$); 2.62-2.68 (m, 1H, $½CH_2CHCO_2H_B$); 2.78-2.84 (m, 1H, $½CH_2CHCO_2H_A$); 3.71-3.76 (m, 1H, $CHCO_2H$); 4.19 (dd, *J* = 12.6, 4.6, 1H, $½CO_2CH_{2\,A}$); 4.23 (dd, *J* = 12.5, 5.8, 1H, $½CO_2CH_{2\,B}$); 4.35-4.40 (m, 1H, $½CO_2CH_2$); 4.74-4.79 (m, 1H, $CO_2CH_2CH_A$); 4.89-4.94 (m, 1H, $CO_2CH_2CH_B$); 5.30-5.38 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture of diastereoisomers A:B (1:1): δ 14.3 (CH_3); 22.8 (CH_2); 24.9 and 24.94 (CH_2); 27.3, 27.37 ($2CH_2CH_{alkene}$); 27.9, 29.2, 29.22, 29.3, 29.5, 29.7, 29.8, 29.9, 31.1 ($9CH_2$); 32.1 (CH_2CHCO_2H); 34.0 and 34.1 (CH_2CO); 46.0 and 46.2 ($CHCO_2H$); 64.4 and 64.9 (CO_2CH_2); 76.7 and 76.8 (CO_2CH_2CH); 129.85 and 129.8 (CH_{alkene}); 130.17 and 130.19 (CH_{alkene}); 171.9, 172.1 (CO_2H, $CO_{lactone}$); 173.4 and 173.6 (CO)

HRMS (ESI, *m/z*): calculated for $C_{23}H_{39}O_4$ ([M-CO_2H]$^-$): 379.2854, found: 379.2861
HPLC (method A, t_R, min): 14.37

(5*S*)-3-Fluoro-5-{[(9*Z*)-octadec-9-enoyloxy]methyl}-2-oxotetrahydrofuran-3-carboxylic acid, 1c

Following the general procedure 4.2.1.8., compound **1c** was obtained from di-*tert*-butyl ester **15** (80 mg, 0.14 mmol) and TFA (0.27 mL, 3.50 mmol) without further purification in quantitative yield.

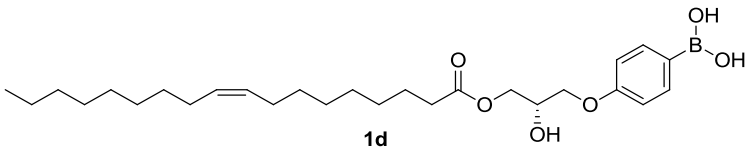


1c

IR (ATR): 3466 (O-H); 1795 (C=O); 1742 (C=O); 1163 (C-F)
^{1}H-NMR ($CDCl_3$, 500 MHz): Mixture of diastereoisomers A:B (1:1): δ 0.88 (t, *J* = 6.8, 3H, CH_3); 1.21-1.37 (m, 20H, 10CH_2); 1.59-1.66 (m, 2H, CH_2CH_2CO); 1.99-2.02 (m, 4H, 2CH_2CH_{alkene}); 2.37 (t, *J* = 7.5, CH_2CO); 2.46-2.55 (m, 1H, ½CH_2CF $_A$); 2.76 (app dd, *J* = 26.1, 7.2, 2H, CH_2CF $_B$); 2.97-3.03 (m, 1H, ½CH_2CF $_A$); 4.24 (dd, *J* = 12.6, 4.8, 1H, ½CO_2CH_2 $_A$); 4.29 (dd, *J* = 12.5, 6.0, 1H, ½CO_2CH_2 $_B$); 4.42-4.46 (m, 1H, ½CO_2CH_2); 4.90-4.99 (m, 1H, CH); 5.31-5.38 (m, 2H, 2CH_{alkene})
^{13}C-NMR ($CDCl_3$, 125 MHz): Mixture of diastereoisomers A:B (1:1): δ 14.2 (CH_3); 22.8, 24.9 (2CH_2); 27.3, 27.4 (2CH_2CH_{alkene}); 29.2, 29.22, 29.3, 29.5, 29.7, 29.81, 29.84, 29.9, 32.0 (9CH_2); 34.0 and 34.08 (CH_2CO); 34.9 (d, *J* = 22.4) and 35.0 (d, *J* = 21.7, CH_2CF); 63.6 and 63.9 (CO_2CH_2); 75.7 (d, *J* = 2.9) and 76.5 (CH); 91.7 (d, *J* = 197.8) and 92.1 (d, *J* = 200.9, CF); 129.9 (CH_{alkene}); 130.16 and 130.18 (CH_{alkene}); 167.5 (d, *J* = 23.3, CO); 167.8 (d, *J* = 24.7, CO); 173.8 and 173.84 (CO)
HRMS (ESI, *m/z*): calculated for $C_{24}H_{38}FO_6$ ([M-H]$^-$): 441.2658, found 441.2667
HPLC (method A, t_R, min): 21.16

[4-({(2*S*)-2-Hydroxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl}oxy)phenyl]boronic acid, 1d

To a solution of compound **17** (30 mg, 54 µmol, 1 equiv) in acetonitrile (0.5 mL), water (3 µL, 0.16 mmol, 3 equiv) and trimethylsilyl chloride (20 µL, 0.16 mmol, 3 equiv) were added and the mixture was stirred for 1 h at room temperature. Then, a saturated aqueous solution of $NaHCO_3$ was added, and the reaction mixture was extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Flash chromatography of the residue (hexane/ethyl acetate, 10:1 to 1:1, followed by ethyl acetate/ethanol, 10:1 to 5:1) afforded compound **1d** in 60% yield.

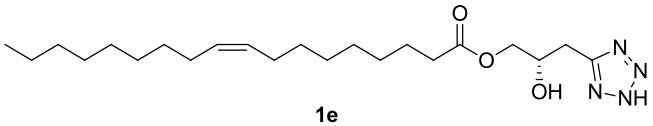


1d

R_f: 0.30 (hexane/ethyl acetate, 1:1)
IR (ATR): 1740 (C=O); 1368 (B-O); 1242 (B-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.5, 3H, CH_3); 1.26-1.30 (m, 20H, $10CH_2$); 1.63-1.67 (m, 2H, CH_2CH_2CO); 1.99-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.38 (t, *J* = 7.5, 2H, CH_2CO); 4.03-4.12 (m, 2H, CH_2OAr); 4.24-4.37 (m, 3H, CH, CO_2CH_2); 5.27-5.40 (m, 2H, $2CH_{alkene}$); 7.02 (d, *J* = 8.5, 2H, $2CH_{Ar}$); 8.16 (d, *J* = 8.4, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2 ($2CH_2$); 29.3 (CH_2); 29.5 ($2CH_2$); 29.7, 29.8, 29.9, 32.1 ($4CH_2$); 34.3 (CH_2CO); 65.4 (CO_2CH_2); 68.6 (CH_2OAr); 68.8 (CH); 114.2 ($2CH_{Ar}$); 129.9, 130.2 ($2CH_{alkene}$); 137.6 ($2CH_{Ar}$); 141.1 (C_{Ar}); 174.2 (CO); CB not observed
$[\alpha]_D^{20}$: limited solubility
HRMS (ESI, *m/z*): calculated for $C_{27}H_{46}BO_6$ ($[M+H]^+$): 477.3387, found 477.3395
HPLC (method A, t_R, min): 22.85

(2*S*)-2-Hydroxy-3-(1*H*-tetrazol-5-yl)propyl (9*Z*)-octadec-9-enoate, 1e

To a solution of compound **18** (64 mg, 0.18 mmol, 1 equiv) in dry DMF (0.3 mL), sodium azide (13 mg, 0.19 mmol, 1.1 equiv) and NH_4Cl (12 mg, 0.23 mmol, 1.3 equiv) were added. The mixture was heated at 160°C under MW irradiation for 45 min. Then, the solvent was removed under reduced pressure. The crude was purified by flash chromatography (hexane to hexane/ethyl acetate, 6:4) afforded compound **1e** in 5% yield.



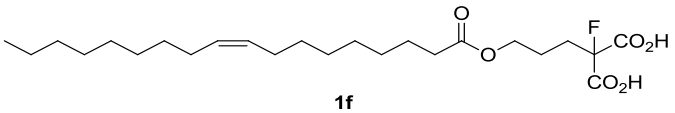
1e

IR (ATR): 3390 (O-H, N-H); 1738 (C=O)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 0.90 (t, *J* = 6.9, 3H, CH_3); 1.29-1.33 (m, 20H, $10CH_2$); 1.58-1.64 (m, 2H, CH_2CH_2CO); 2.01-2.05 (m, 4H, $2CH_2CH_{alkene}$); 2.34 (t, *J* = 7.4, 2H, CH_2CO); 3.07 (dd, *J* = 14.9, 8.2, 1H, ½CH_2CN); 3.18 (dd, *J* = 14.9, 4.6, 1H, ½CH_2CN); 4.06-4.12 (m, 2H, CO_2CH_2); 4.17-4.22 (m, 1H, CH); 5.31-5.37 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 14.4 (CH_3); 23.7, 25.9 ($2CH_2$); 28.1 ($2CH_2CH_{alkene}$); 29.3 (CH_2CN); 30.18, 30.19, 30.3, 30.33, 30.4, 30.6, 30.8, 30.84, 33.1 ($9CH_2$); 34.8 (CH_2CO); 68.2 (CO_2CH_2); 68.3 (CH); 130.8, 130.9 ($2CH_{alkene}$); 155.5 (CN); 175.2 (CO)
$[\alpha]_D^{20}$: +31.7 (c = 0.09, methanol)

HRMS (MALDI, *m/z*): calculated for $C_{22}H_{40}N_4NaO_3$ ($[M+Na]^+$): 431.2998, found: 431.1931
HPLC (method A, t_R, min): 19.45

Fluoro{3-[(9*Z*)-octadec-9-enoyloxy]propyl}propanedioic acid, 1f

Following the general procedure 4.2.1.8., compound **1f** was obtained from di-*tert*-butyl ester **24** (61 mg, 0.11 mmol) and TFA (0.21 mL, 2.75 mmol) without further purification in 90% yield.



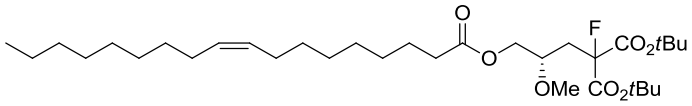
1f

IR (ATR): 1738 (C=O); 1166 (C-F)
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 0.88 (t, *J* = 6.6, 3H, CH_3); 1.18-1.39 (m, 20H, $10CH_2$); 1.54-1.67 (m, 2H, CH_2CH_2CO); 1.70-1.85 (m, 2H, CH_2CH_2CF); 1.93-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.20-2.45 (m, 2H, CH_2CF); 2.41 (t, *J* = 7.6, 2H, CH_2CO); 4.02-4.18 (m, 2H, CO_2CH_2); 5.27-5.41 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (DMSO-d_6, 75 MHz): δ 14.1 (CH_3); 22.7, 24.9 ($2CH_2$); 24.93 (d, *J* = 2.8, CH_2CH_2CF); 27.2, 27.23 ($2CH_2CH_{alkene}$); 29.1, 29.2 ($2CH_2$); 29.3 ($2CH_2$); 29.5, 29.7, 29.74, 29.8 ($4CH_2$); 31.1 (d, *J* = 21.6, CH_2CF); 31.9 (CH_2); 34.3 (CH_2CO); 63.29 (CO_2CH_2); 129.7, 130.0 ($2CH_{alkene}$); 174.7 (CO); $2CO_2H$ and CF not observed
HRMS (ESI, *m/z*): calculated for $C_{24}H_{40}FO_6$ ($[M-H]^-$): 443.2814, found: 443.2824
HPLC (method A, t_R, min): 16.75

4.2.3. Synthesis of final compound **1g**

Di-*tert*-butyl fluoro{(2*S*)-2-methoxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl} propanedioate, 24

Following the general procedure 4.2.1.9., compound **24** was obtained from **15** (90 mg, 0.16 mmol) in 38% yield. Chromatography: hexane/ethyl acetate, 30:1 to 5:1.



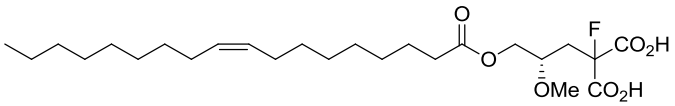
24

R_f: 0.41 (hexane/ethyl acetate, 8:2)
IR (ATR): 1769 (C=O); 1165 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.5, 3H, CH_3); 1.26-1.29 (m, 20H, $10CH_2$); 1.48 (s, 9H, $3CH_3$); 1.49 (s, 9H, $3CH_3$); 1.59-1.64 (m, 2H, CH_2CH_2CO); 1.99-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.16-2.48 (m, 2H, CH_2CF); 2.32 (t, *J* = 7.5, 2H, CH_2CO); 3.29 (s, 3H, OCH_3); 3.57-3.64 (m, 1H, CH); 4.10 (ABX system, *J* = 11.6, 4.8, 4.6, 2H, CO_2CH_2); 5.28-5.39 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.0 ($2CH_2$); 27.3, 27.34 ($2CH_2CH_{alkene}$); 27.8 ($3CH_3$); 27.9 ($3CH_3$); 29.2 ($2CH_2$); 29.3 (CH_2); 29.5 ($2CH_2$); 29.7, 29.8, 29.9, 32.0 ($4CH_2$); 34.3 (CH_2CO); 36.7 (d, *J* = 21.4, CH_2CF); 57.9 (OCH_3); 64.9 (CO_2CH_2); 73.9 (d, *J* = 3.3, CH); 83.2, 83.6 ($2C(CH_3)_3$); 92.5 (d, *J* = 195.3, CF); 129.9, 130.1 ($2CH_{alkene}$); 165.1 (d, *J* = 27.4, CO_2tBu); 165.2 (d, *J* = 23.8, CO_2tBu); 173.7 (CO)
$[\alpha]_D^{20}$: +4.1 (c = 0.75, $CHCl_3$)
MS (ESI, *m/z*): 609.4 $[M+Na]^+$

Fluoro{(2*S*)-2-methoxy-3-[(9*Z*)-octadec-9-enoyloxy]propyl}propanedioic acid, 1g

Following the general procedure 4.2.1.8., compound **1g** was obtained from di-*tert*-butyl ester **24** (10 mg, 17 µmol) and TFA (98 µL, 1.28 mmol) without further purification in 95% yield.



1g

IR (ATR): 3525 (O-H); 1742 (C=O); 1260 (C-F)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 0.90 (t, *J* = 7.1, 3H, CH_3); 1.29-1.33 (m, 20H, $10CH_2$); 1.61-1.63 (m, 2H, CH_2CH_2CO); 2.02-2.05 (m, 4H, $2CH_2CH_{alkene}$), 2.24-2.30 (m, 1H, ½CH_2CF); 2.35 (t, *J* = 7.4, 2H, CH_2CO); 2.45-2.52 (m, 1H, ½CH_2CF); 3.33 (s, 3H, OCH_3); 3.63-3.65 (m, 1H, CH); 4.04 (dd, *J* = 11.7, 4.9, 1H, ½CO_2CH_2); 4.25 (dd, *J* = 11.7, 3.9, 1H, ½CO_2CH_2); 5.32-5.37 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 14.5 (CH_3); 23.8, 26.0 ($2CH_2$); 28.1 ($2CH_2CH_{alkene}$); 30.2 ($2CH_2$); 30.3, 30.4, 30.5, 30.6, 30.8, 30.9, 33.1 ($7CH_2$); 34.9 (CH_2CO); 38.4 (d, *J* = 20.1, CH_2CF); 58.1 (OCH_3); 65.9 (CO_2CH_2); 76.0 (CH); 94.4 (d, *J* = 192.5, CF); 130.8, 130.9 ($2CH_{alkene}$); 170.9 (br s, $2CO_2H$); 175.2 (CO)
$[\alpha]_D^{20}$: +29.5 (c = 0.20, methanol)
HRMS (ESI, *m/z*): calculated for $C_{25}H_{42}FO_7$ ($[M-H]^-$): 473.2915, found: 473.2922
HPLC (method A, t_R, min): 14.51

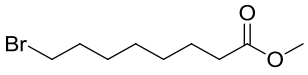
4.2.4. Synthesis of final compounds **2a**-**e**

4.2.4.1. Synthesis of non-commercial fatty acids **32**-**34**

Methyl 8-bromooctanoate, 25

To a solution of 8-bromooctanoic acid (2.0 g, 8.96 mmol, 1 equiv) in methanol (10 mL), concentrated H_2SO_4 (0.2 mL) was added and the reaction mixture was refluxed overnight. Afterward, the solvent was evaporated and the residue was dissolved in ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced

pressure to yield ester **25** in quantitative yield, which was used in the next step without further purification. The spectroscopic data correspond with those previously reported.[105]

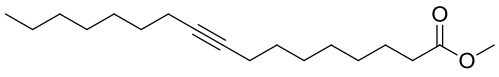


25

R_f: 0.23 (hexane/ethyl acetate, 20:1)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.30 (m, 4H, $2CH_2$); 1.40-1.45 (m, 2H, CH_2); 1.57-1.67 (m, 2H, CH_2); 1.84 (qt, *J* = 7.5, 2H, CH_2CH_2CO); 2.30 (t, *J* = 7.5, 2H, CH_2CO); 3.39 (t, *J* = 6.8, 2H, CH_2Br); 3.66 (s, 3H, CH_3)

Methyl heptadec-9-ynoate, 26

Following the general procedure 4.2.1.10., alkyne **26** was obtained from 1-nonyne (0.27 mL, 1.64 mmol) and methyl 8-bromooctanoate **25** (300 mg, 1.26 mmol) in 36% yield. Chromatography: hexane.

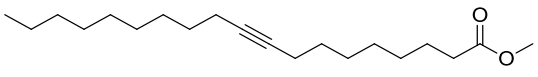


26

R_f: 0.32 (hexane/ethyl acetate, 20:1)
IR (ATR): 1740 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.5, 3H, CH_3); 1.28-1.38 (m, 14H, $7CH_2$); 1.42-1.49 (m, 4H, $2CH_2$); 1.58-1.65 (m, 2H, CH_2CH_2CO); 2.13 (t, *J* = 6.9, 4H, $2CH_2C_{alkyne}$); 2.30 (t, *J* = 7.5, 2H; CH_2CO); 3.66 (s, 3H, OCH_3)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 18.87, 18.9 ($2CH_2C_{alkyne}$); 22.8, 25.1, 28.8, 29.9 ($4CH_2$); 29.0 ($2CH_2$); 29.2, 29.22, 29.3, 31.9 ($4CH_2$); 34.2 (CH_2CO); 51.6 (OCH_3); 80.2, 80.5 ($2C_{alkyne}$); 174.4 (CO)

Methyl nonadec-9-ynoate, 27

Following the general procedure 4.2.1.10., alkyne **27** was obtained from 1-undecyne (0.33 mL, 1.64 mmol) and methyl 8-bromooctanoate **25** (300 mg, 1.26 mmol) in 38% yield. Chromatography: hexane.



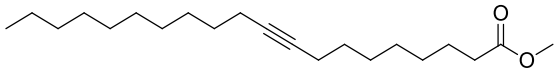
27

R_f: 0.36 (hexane/ethyl acetate, 20:1)
IR (ATR): 1742 (C=O)

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.27-1.51 (m, 22H, $11CH_2$); 1.57-1.67 (m, 2H, CH_2CH_2CO); 2.11-2.16 (m, 4H, $2CH_2C_{alkyne}$); 2.30 (t, *J* = 7.5, 2H, CH_2CO), 3.66 (s, 3H, OCH_3)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 18.88, 18.9 ($2CH_2C_{alkyne}$); 22.8, 25.1, 28.8, 28.9, 29.0, 29.2, 29.22 ($7CH_2$); 29.3 ($2CH_2$); 29.5, 29.7, 32.1 ($3CH_2$); 34.2 (CH_2CO); 51.6 (OCH_3); 80.2, 80.5 ($2C_{alkyne}$), 174.4 (CO)
MS (ESI, *m/z*): 309.3 $[M+H]^+$

Methyl icos-9-ynoate, 28

Following the general procedure 4.2.1.10., alkyne **28** was obtained from 1-dodecyne (0.32 mL, 1.64 mmol) and methyl 8-bromooctanoate **25** (300 mg, 1.26 mmol) in 44% yield. Chromatography: hexane.

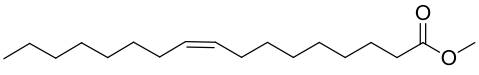


28

R_f: 0.48 (hexane/ethyl acetate, 20:1)
IR (ATR): 1740 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.26-1.40 (m, 20H, $10CH_2$); 1.42-1.49 (m, 4H, $2CH_2$); 1.57-1.67 (m, 2H, CH_2CH_2CO); 2.13 (t, *J* = 6.9, 4H, $2CH_2C_{alkyne}$); 2.30 (t, *J* = 7.5, 2H, CH_2CO); 3.66 (s, 3H, OCH_3)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 18.88, 18.9 ($2CH_2C_{alkyne}$); 22.8, 25.1, 28.8, 28.9, 29.0, 29.2, 29.22 ($7CH_2$); 29.3 ($2CH_2$); 29.5, 29.7, 29.74, 32.1 ($4CH_2$); 34.2 (CH_2CO); 51.6 (OCH_3); 80.2, 80.5 ($2C_{alkyne}$); 174.4 (CO)

Methyl (9*Z*)-heptadec-9-enoate, 29

Following the general procedure 4.2.1.11., alkene **29** was obtained from alkyne **26** (126 mg, 0.45 mmol) in 91% yield.

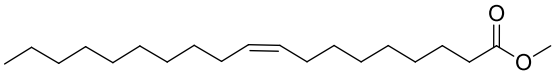


29

R_f: 0.32 (hexane/ethyl acetate, 20:1)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.6, 3H, CH_3); 1.27-1.29 (m, 18H, $9CH_2$); 1.59-1.63 (m, 2H, CH_2CH_2CO); 1.97-2.06 (m, 4H, $2CH_2CH_{alkene}$); 2.30 (t, *J* = 7.5, 2H, CH_2CO); 3.66 (s, 3H, OCH_3); 5.28-5.39 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.26, 29.29, 29.3, 29.4, 29.8, 29.9, 32.0 ($8CH_2$); 34.2 (CH_2CO); 51.6 (OCH_3); 129.9, 130.1 ($2CH_{alkene}$); 174.5 (CO)

Methyl (9*Z*)-nonadec-9-enoate, 30

Following the general procedure 4.2.1.11., alkene **30** was obtained from alkyne **27** (120 mg, 0.39 mmol) in 92% yield.

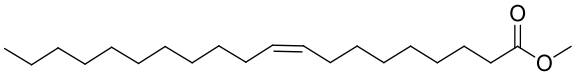


30

R_f: 0.36 (hexane/ethyl acetate, 20:1)
IR (ATR): 1742 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.26-1.30 (m, 22H, $11CH_2$); 1.58-1.64 (m, 2H, CH_2CH_2CO); 1.97-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.30 (t, *J* = 7.5, 2H, CH_2CO); 3.66 (s, 3H, OCH_3); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.3, 29.31, 29.4, 29.49, 29.7, 29.8, 29.84, 29.9, 32.1 ($10CH_2$); 34.3 (CH_2CO); 51.6 (OCH_3); 129.9, 130.2 ($2CH_{alkene}$); 174.5 (CO)
MS (ESI, *m/z*): 311.3 $[M+H]^+$

Methyl (9*Z*)-icos-9-enoate, 31

Following the general procedure 4.2.1.11., alkene **31** was obtained from alkyne **28** (128 mg, 0.40 mmol) in 89% yield.

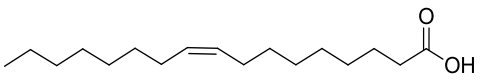


31

R_f: 0.48 (hexane/ethyl acetate, 20:1)
IR (ATR): 1743 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.26-1.30 (m, 24H, $12CH_2$); 1.57-1.64 (m, 2H, CH_2CH_2CO); 1.97-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.30 (t, *J* = 7.5, 2H, CH_2CO); 3.66 (s, 3H, OCH_3); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.28, 29.3, 29.4, 29.5, 29.7, 29.8, 29.81, 29.84, 29.9, 32.1 ($11CH_2$); 34.3 (CH_2CO); 51.6 (OCH_3); 129.9, 130.2 ($2CH_{alkene}$); 174.5 (CO)

(9*Z*)-Heptadec-9-enoic acid, 32

Following the general procedure 4.2.1.12., carboxylic acid **32** was obtained from ester **29** (115 mg, 0.41 mmol) in quantitative yield.

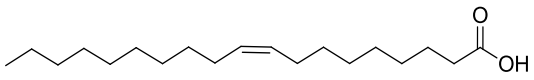


32

R_f: 0.43 (hexane/ethyl acetate, 1:1)
IR (ATR): 3252 (O-H); 1710 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.27-1.31 (m, 18H, $9CH_2$); 1.58-1.66 (m, 2H, CH_2CH_2CO); 1.98-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 24.8 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.21, 29.3, 29.37, 29.4, 29.8, 29.9, 32.0 ($8CH_2$); 34.2 (CH_2CO); 129.9, 130.2 ($2CH_{alkene}$); 180.0 (CO)
MS (ESI, *m/z*): 267.1 $[M-H]^-$

(9*Z*)-Nonadec-9-enoic acid, 33

Following the general procedure 4.2.1.12., carboxylic acid **33** was obtained from ester **30** (111 mg, 0.36 mmol) in quantitative yield.

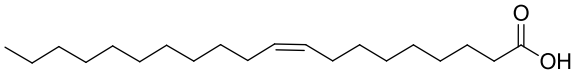


33

R_f: 0.45 (hexane/ethyl acetate, 1:1)
IR (ATR): 1710 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.26-1.31 (m, 22H, $11CH_2$); 1.58-1.68 (m, 2H, CH_2CH_2CO); 1.98-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 24.8 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.22, 29.3, 29.5, 29.51, 29.7, 29.8, 29.83, 29.9, 32.1 ($10CH_2$); 34.2 (CH_2CO); 129.9, 130.2 ($2CH_{alkene}$); 180.2 (CO)

(9*Z*)-Icos-9-enoic acid, 34

Following the general procedure 4.2.1.12., carboxylic acid **34** was obtained from ester **31** (114 mg, 0.35 mmol) in quantitative yield.



34

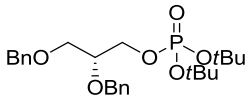
R_f: 0.46 (hexane/ethyl acetate, 1:1)
IR (ATR): 1711 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.26-1.31 (m, 24H, $12CH_2$); 1.56-1.68 (m, 2H, CH_2CH_2CO); 1.94-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.9, 24.8 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.22, 29.3, 29.5, 29.51, 29.7, 29.8 ($7CH_2$); 29.82 ($2CH_2$); 29.9, 32.1 ($2CH_2$); 34.1 (CH_2CO); 129.9, 130.2 ($2CH_{alkene}$); 180.0 (CO)

MS (ESI, *m/z*): 309.2 $[M-H]^-$

4.2.4.2. Synthesis of phosphorylated diol **36**

(2*R*)-2,3-Bis(benzyloxy)propyl di-*tert*-butyl phosphate, 35

Following the general procedure 4.2.1.13., compound **35** was obtained from (*S*)-2,3-bis(benzyloxy)propan-1-ol (416 mg, 1.53 mmol) and di-*tert*-butyl-*N*,*N*-diisopropylphosphoramidite (0.85 mL, 3.06 mmol) in 38% yield. Chromatography: hexane to hexane/ethyl acetate, 1:1.

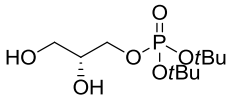


35

R_f: 0.36 (hexane/ethyl acetate, 1:1)
IR (ATR): 1265 (P=O); 996 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.46 (s, 18H, $6CH_3$); 3.56-3.66 (m, 2H, CH_2OBn); 3.83 (qt, *J* = 5.1, 1H, CH); 4.00-4.15 (m, 2H, CH_2OP); 4.54 (s, 2H, PhCH_2); 4.59-4.67 (m, 2H, PhCH_2); 7.25-7.37 (m, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 29.9 ($3CH_3$); 30.0 ($3CH_3$); 66.1 (d, *J* = 6.4, CH_2OP); 69.8 (CH_2OBn); 72.4 (PhCH_2); 73.5 (PhCH_2); 76.9 (d, *J* = 8.6, CH); 82.4 (d, *J* = 7.5, C); 82.5 (d, *J* = 7.4, C); 127.7 ($4CH_{Ar}$); 127.9 ($2CH_{Ar}$); 128.4 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 138.3, 138.5 ($2C_{Ar}$)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -6.82
$[\alpha]_D^{20}$: +1.6 (c = 1.00, $CHCl_3$)
MS (ESI, *m/z*): 487.1 $[M+Na]^+$

Di-*tert*-butyl (2*R*)-2,3-dihydroxypropyl phosphate, 36

Following the general procedure 4.2.1.1., diol **36** was obtained from **35** (220 mg, 0.47 mmol) at 60ºC in quantitative yield.



36

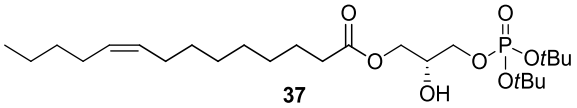
R_f: 0.06 (hexane/ethyl acetate, 1:1)
IR (ATR): 3345 (O-H); 1025 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.47 (s, 18H, $6CH_3$); 3.60-3.73 (m, 2H, CH_2OH); 3.86-3.92 (m, 3H, CH, 2OH); 4.02 (dd, *J* = 9.1, 5.3, 2H, CH_2OP)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 30.1 ($3CH_3$); 30.2 ($3CH_3$); 63.7 (CH_2OH); 69.2 (d, *J* = 6.8, CH_2OP); 71.9 (d, *J* = 10.1, CH); 84.5 (d, *J* = 7.7, 2C)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -6.03

$[\alpha]_D^{20}$: -1.5 (c = 1.70, methanol)
MS (ESI, *m/z*): 307.1 $[M+Na]^+$

4.2.4.3. Synthesis of esters **37**-**41**

(2*R*)-3-[(Di-*tert*-butoxyphosphoryl)oxy]-2-hydroxypropyl (9*Z*)-tetradec-9-enoate, 37

Following the general procedure 4.2.1.14., ester **37** was obtained from myristoleic acid (24 µL, 95 µmol) and diol **36** (54 mg, 0.19 mmol) in 28% yield. Chromatography: dichloromethane to dichloromethane/methanol, 95:5.

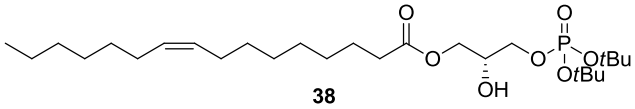


37

R_f: 0.88 (dichloromethane/methanol, 95:5)
IR (ATR): 3365 (O-H); 1739 (C=O); 1257 (P=O); 1000 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.89 (t, *J* = 7.0, 3H, CH_3); 1.24-1.34 (m, 12H, $6CH_2$); 1.49 (s, 18H, $6CH_3$); 1.57-1.64 (m, 2H, CH_2CH_2CO); 1.94-2.02 (m, 4H, $2CH_2CH_{alkene}$); 2.33 (t, *J* = 7.6, 2H, CH_2CO); 3.95-4.13 (m, 3H, CH, CH_2OP); 4.15 (app d, *J* = 4.7, 2H, CO_2CH_2); 5.27-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.1 (CH_3); 22.5, 25.0 ($2CH_2$); 27.1, 27.3 ($2CH_2CH_{alkene}$); 29.2, 29.23, 29.3, 29.8 ($4CH_2$); 29.9 ($3CH_3$); 30.0 ($3CH_3$); 32.1 (CH_2); 34.3 (CH_2CO); 64.7 (CO_2CH_2); 68.3 (d, *J* = 5.8, CH_2OP); 69.1 (d, *J* = 5.0, CH); 83.4 (d, *J* = 7.5, 2C); 129.9, 130.1 ($2CH_{alkene}$); 173.9 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ -5.11
$[\alpha]_D^{20}$: +2.6 (c = 0.50, methanol)
MS (ESI, *m/z*): 515.2 $[M+Na]^+$

(2*R*)-3-[(Di-*tert*-butoxyphosphoryl)oxy]-2-hydroxypropyl (9*Z*)-hexadec-9-enoate, 38

Following the general procedure 4.2.1.14., ester **38** was obtained from palmitoleic acid (66 µL, 0.23 mmol) and diol **36** (133 mg, 0.47 mmol) in 49% yield. Chromatography: hexane/ethyl acetate, 40:1 to 1:1.



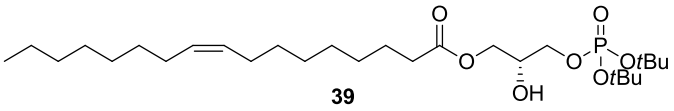
38

R_f: 0.38 (hexane/ethyl acetate, 1:1)
IR (ATR): 3363 (O-H); 1739 (C=O); 1251 (P=O); 1000 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.7, 3H, CH_3); 1.28 (m, 16H, $8CH_2$); 1.48 (s, 18H, $6CH_3$); 1.56-1.69 (m, 2H, CH_2CH_2CO); 1.96-2.00 (m, 4H, $2CH_2CH_{alkene}$); 2.32 (t,

J = 7.6, 2H, CH_2CO); 3.85 (br s, 1H, OH); 3.95-4.09 (m, 3H, CH, CH_2OP); 4.14 (app d, *J* = 4.6, 2H, CO_2CH_2); 5.27-5.38 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 22.8, 25.0 ($2CH_2$); 27.3, 27.33 ($2CH_2CH_{alkene}$); 29.1 (CH_2); 29.2 ($2CH_2$); 29.3, 29.8, 29.84 ($3CH_2$); 29.9 ($3CH_3$); 30.0 ($3CH_3$); 31.9 (CH_2); 34.2 (CH_2CO); 64.7 (CO_2CH_2); 68.3 (d, *J* = 6.0, CH_2OP); 69.0 (d, *J* = 5.2, CH); 83.4 (d, *J* = 7.5, 2C); 129.9, 130.1 ($2CH_{alkene}$); 173.9 (CO)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -5.23
$[\alpha]_D^{20}$: -1.9 (c = 1.21, $CHCl_3$)
MS (ESI, *m/z*): 543.3 $[M+Na]^+$

(2*R*)-3-[(Di-*tert*-butoxyphosphoryl)oxy]-2-hydroxypropyl (9*Z*)-heptadec-9-enoate, 39

Following the general procedure 4.2.1.14., ester **39** was obtained from carboxylic acid **32** (92 mg, 0.34 mmol) and diol **36** (195 mg, 0.69 mmol) in 40% yield. Chromatography: hexane/ethyl acetate, 20:1 to 2:1.

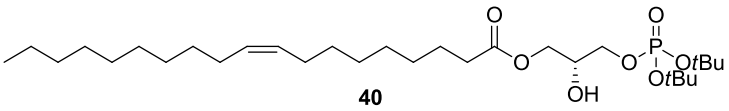


39

R_f: 0.36 (hexane/ethyl acetate, 1:1)
IR (ATR): 3366 (O-H); 1739 (C=O); 1647 (C=C); 1259 (P=O); 1007 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.21-1.30 (m, 18H, $9CH_2$); 1.50 (s, 18H, $6CH_3$); 1.60-1.64 (m, 2H, CH_2CH_2CO); 1.97-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.34 (t, *J* = 7.6, 2H, CH_2CO); 3.71 (br s, 1H, OH); 3.98-4.11 (m, 3H, CH, CH_2OP); 4.16 (app d, *J* = 4.9, 2H, CO_2CH_2); 5.28-5.39 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.1 (CH_3); 22.7, 24.9 ($2CH_2$); 27.2, 27.24 ($2CH_2CH_{alkene}$); 29.1 ($2CH_2$); 29.2, 29.25, 29.3 ($3CH_2$); 29.7 ($2CH_2$); 29.8 ($3CH_3$); 29.9 ($3CH_3$); 31.9 (CH_2); 34.1 (CH_2CO); 64.5 (CO_2CH_2); 68.3 (d, *J* = 5.4, CH_2OP); 69.1 (d, *J* = 4.8, CH); 83.3 (d, *J* = 7.8, 2C); 129.8, 130.0 ($2CH_{alkene}$); 173.8 (CO)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -5.01
$[\alpha]_D^{20}$: +3.5 (c = 0.77, methanol)
MS (ESI, *m/z*): 557.3 $[M+Na]^+$

(2*R*)-3-[(di-*tert*-butoxyphosphoryl)oxy]-2-hydroxypropyl (9*Z*)-nonadec-9-enoate, 40

Following the general procedure 4.2.1.14., ester **40** was obtained from carboxylic acid **33** (73 mg, 0.25 mmol) and diol **36** (140 mg, 0.49 mmol) in 17% yield. Chromatography: hexane/ethyl acetate, 20:1 to 2:1.

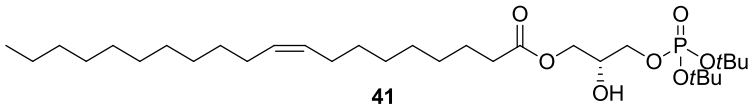


40

R_f: 0.37 (hexane/ethyl acetate, 1:1)
IR (ATR): 3359 (O-H); 1738 (C=O); 1650 (C=C); 1252 (P=O); 1010 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.7, 3H, CH_3); 1.26-1.29 (m, 22H, $11CH_2$); 1.49 (s, 18H, $6CH_3$); 1.59-1.64 (m, 2H, CH_2CH_2CO); 1.97-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.33 (t, *J* = 7.6, 2H, CH_2CO); 3.76 (br s, 1H, OH); 3.95-4.10 (m, 3H, CH, CH_2OP); 4.15 (app d, *J* = 4.9, 2H, CO_2CH_2); 5.28-5.39 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.1 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2 ($2CH_2$); 29.3, 29.4, 29.5, 29.7, 29.74, 29.8, 29.9 ($7CH_2$); 29.93 ($3CH_3$); 30.0 ($3CH_3$); 32.0 (CH_2); 34.1 (CH_2CO); 64.7 (CO_2CH_2); 68.3 (d, *J* = 6.0, CH_2OP); 69.1 (d, *J* = 5.0, CH); 83.4 (d, *J* = 7.5, 2C); 129.9, 130.1 ($2CH_{alkene}$); 173.9 (CO)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -5.15
$[\alpha]_D^{20}$: -1.7 (c = 1.18, $CHCl_3$)
MS (ESI, *m/z*): 449.4 $[M-2t\text{Bu}+H]^+$; 450.4 $[M-2t\text{Bu}+2H]^+$

(2*R*)-3-[(Di-*tert*-butoxyphosphoryl)oxy]-2-hydroxypropyl (9*Z*)-icos-9-enoate, 41

Following the general procedure 4.2.1.14., ester **41** was obtained from carboxylic acid **34** (65 mg, 0.21 mmol) and diol **36** (121 mg, 0.42 mmol) in 12% yield. Chromatography: hexane/ethyl acetate, 20:1 to 2:1.



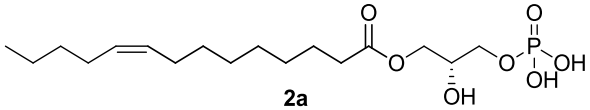
41

R_f: 0.43 (hexane/ethyl acetate, 1:1)
IR (ATR): 3370 (O-H); 1740 (C=O); 1258 (P=O); 1000 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.7, 3H, CH_3); 1.26-1.30 (m, 24H, $12CH_2$); 1.50 (s, 18H, $6CH_3$); 1.60-1.65 (m, 2H, CH_2CH_2CO), 1.97-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.33 (t, *J* = 7.6, 2H, CH_2CO); 3.72 (d, *J* = 4.1, 1H, OH); 3.98-4.11 (m, 3H, CH, CH_2OP); 4.16 (app d, *J* = 4.9, 2H, CO_2CH_2); 5.28-5.39 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.0 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2 ($2CH_2$); 29.3, 29.4, 29.5, 29.7, 29.78, 29.8, 29.84, 29.9 ($8CH_2$); 30.0 ($6CH_3$); 32.1 (CH_2); 34.3 (CH_2CO); 64.7 (CO_2CH_2); 68.4 (d, *J* = 5.5, CH_2OP); 69.2 (d, *J* = 4.6, CH); 83.4 (d, *J* = 7.2, 2C); 129.9, 130.2 ($2CH_{alkene}$); 173.9 (CO)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -5.01
$[\alpha]_D^{20}$: +3.4 (c = 0.92, methanol)
MS (MALDI, *m/z*): 599.4 $[M+Na]^+$

4.2.4.4. Synthesis of final compounds **2a**-**e**

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl (9*Z*)-tetradec-9-enoate, 2a

Following the general procedure 4.2.1.8., compound **2a** was obtained from di-*tert*-butyl phosphate **37** (7 mg, 14 µmol) and TFA (27 µL, 0.35 mmol) without further purification in 99% yield.

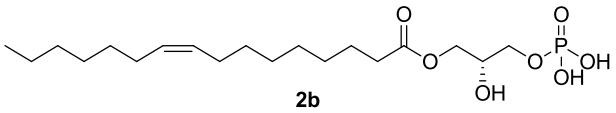


2a

IR (ATR): 3375 (O-H); 1737 (C=O); 1182 (P=O); 1058 (P-OH)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 0.91 (t, *J* = 7.0, 3H, CH_3); 1.29-1.30 (m, 12H, $6CH_2$); 1.60-1.63 (m, 2H, CH_2CH_2CO); 2.02-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.36 (t, *J* = 7.4, 2H, CH_2CO); 3.96-3.98 (m, 3H, CH, CH_2OP); 4.07-4.17 (m, 2H, CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 14.3 (CH_3); 23.4, 26.0 ($2CH_2$); 27.9, 28.1 ($2CH_2CH_{alkene}$); 30.1, 30.2, 30.3, 30.8, 33.1 ($5CH_2$); 34.9 (CH_2CO); 66.2 (CO_2CH_2); 67.6 (d, *J* = 5.2, CH_2OP); 69.8 (d, *J* = 6.9, CH); 130.8, 130.83 ($2CH_{alkene}$); 175.4 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.23
$[\alpha]_D^{20}$: limited solubility
HRMS (ESI, *m/z*): calculated for $C_{17}H_{32}O_7P$ ($[M-H]^-$): 379.2891, found: 379.2848
HPLC (method A, t_R, min): 9.81 min

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl (9*Z*)-hexadec-9-enoate, 2b

Following the general procedure 4.2.1.8., compound **2b** was obtained from di-*tert*-butyl phosphate **38** (24 mg, 46 µmol) and TFA (88 µL, 1.15 mmol) without further purification in 90% yield.



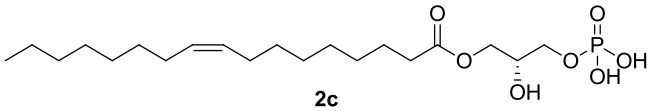
2b

IR (ATR): 3392 (O-H); 1737 (C=O); 1176 (P=O); 1057 (P-OH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 0.90 (t, *J* = 6.7, 3H, CH_3); 1.29-1.33 (m, 16H, $8CH_2$); 1.60-1.64 (m, 2H, CH_2CH_2CO); 2.00-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.36 (t, *J* = 7.5, 2H, CH_2CO); 3.92-4.04 (m, 3H, CH, CH_2OP); 4.15 (ABX system, *J* = 11.4, 5.5, 4.2, 2H, CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 14.5 (CH_3); 23.7, 26.0 ($2CH_2$); 28.1, 28.2 ($2CH_2CH_{alkene}$); 30.1 (CH_2); 30.2 ($2CH_2$); 30.3, 30.8, 30.84, 32.9 ($4CH_2$); 34.9 (CH_2CO); 66.2 (CO_2CH_2); 67.6 (d, *J* = 5.3, CH_2OP); 69.8 (d, *J* = 8.0, CH); 130.8, 130.9 ($2CH_{alkene}$); 175.4 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.79

$[\alpha]_D^{20}$: limited solubility
HRMS (MALDI, *m/z*): calculated for $C_{19}H_{37}NaO_7P$ ($[M+Na]^+$): 431.2175, found: 431.2169
HPLC (method A, t_R, min): 10.06

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl (9*Z*)-heptadec-9-enoate, 2c

Following the general procedure 4.2.1.8., compound **2c** was obtained from di-*tert*-butyl phosphate **39** (6 mg, 12 µmol) and TFA (22 µL, 0.30 mmol) without further purification in 99% yield.

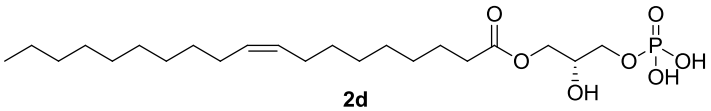


2c

IR (ATR): 3347 (O-H); 1737 (C=O); 1652 (C=C); 1186 (P=O); 1082 (P-OH)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 0.90 (t, *J* = 6.7, 3H, CH_3); 1.29-1.33 (m, 18H, $9CH_2$); 1.60-1.64 (m, 2H, CH_2CH_2CO); 2.01-2.04 (m, 4H, $2CH_2CH_{alkene}$), 2.36 (t, *J* = 7.4, 2H, CH_2CO); 3.89-3.91 (m, 2H, CH_2OP); 3.95-3.98 (m, 1H, CH); 4.11 (dd, *J* = 11.4, 6.2, 1H, ½CO_2CH_2); 4.18 (dd, *J* = 11.4, 4.3, 1H, ½CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 14.5 (CH_3); 23.7, 26.0 ($2CH_2$); 28.1 ($2CH_2CH_{alkene}$); 30.2, 30.22, 30.3, 30.34, 30.8, 30.82, 30.9, 33.1 ($8CH_2$); 34.9 (CH_2CO); 66.4 (CO_2CH_2); 67.2 (d, *J* = 5.2, CH_2OP); 70.1 (d, *J* = 7.9, CH); 130.8, 130.9 ($2CH_{alkene}$); 175.4 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 4.34
$[\alpha]_D^{20}$: limited solubility
HRMS (MALDI, *m/z*): calculated for $C_{20}H_{39}NaO_7P$ ($[M+Na]^+$): 445.2331, found: 445.2311
HPLC (method A, t_R, min): 10.42

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl (9*Z*)-nonadec-9-enoate, 2d

Following the general procedure 4.2.1.8., compound **2d** was obtained from di-*tert*-butyl phosphate **40** (24 mg, 43 µmol) and TFA (82 µL, 1.08 mmol) without further purification in 99% yield.



2d

IR (ATR): 3360 (O-H); 1738 (C=O); 1181 (P=O); 1060 (P-OH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 0.90 (t, *J* = 6.6, 3H, CH_3); 1.29-1.33 (m, 22H, $11CH_2$); 1.59-1.64 (m, 2H, CH_2CH_2CO); 2.02-2.04 (m, 4H, $2CH_2CH_{alkene}$); 2.35 (t, *J* =

7.5, 2H, CH_2CO); 3.91-4.05 (m, 3H, CH, CH_2OP); 4.15 (ABX system, *J* = 11.4, 5.6, 4.3, 2H, CO_2CH_2); 5.29-5.40 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR (methanol-d_4, 75 MHz): δ 14.4 (CH_3); 23.7, 26.0 ($2CH_2$); 28.1 ($2\underline{C}H_2CH_{alkene}$); 30.2 ($2CH_2$); 30.3 ($2CH_2$); 30.5, 30.6, 30.7 ($3CH_2$); 30.8 ($2CH_2$); 33.1 ($CH_2$); 34.9 ($\underline{C}H_2CO$); 66.3 ($CO_2\underline{C}H_2$); 67.5 (d, *J* = 4.8, CH_2OP); 69.9 (d, *J* = 7.7, CH); 130.8, 130.9 ($2CH_{alkene}$); 175.4 (CO)

^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.88

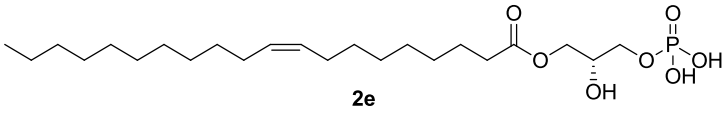
$[\alpha]_D^{20}$: limited solubility

HRMS (MALDI, *m/z*): calculated for $C_{22}H_{43}NaO_7P$ ($[M+Na]^+$): 473.2644, found: 473.2642

HPLC (method A, t_R, min): 11.30

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl (9*Z*)-icos-9-enoate, 2e

Following the general procedure 4.2.1.8., compound **2e** was obtained from di-*tert*-butyl phosphate **41** (9 mg, 16 µmol) and TFA (30 µL, 0.40 mmol) without further purification in 92% yield.



2e

IR (ATR): 3350 (O-H); 1738 (C=O); 1179 (P=O); 1059 (P-OH)

^{1}H-NMR (methanol-d_4, 700 MHz): δ 0.90 (t, *J* = 7.0, 3H, CH_3); 1.29-1.33 (m, 24H, $12CH_2$); 1.60-1.63 (m, 2H, $C\underline{H}_2CH_2CO$); 2.02-2.05 (m, 4H, $2C\underline{H}_2CH_{alkene}$); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 3.94 (app t, *J* = 5.7, 2H, CH_2OP); 3.97-4.00 (m, 1H, CH); 4.11 (dd, *J* = 11.4, 6.0, 1H, ½CO_2CH_2); 4.18 (dd, *J* = 11.4, 4.3, 1H, ½CO_2CH_2); 5.32-5.37 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR (methanol-d_4, 175 MHz): δ 14.5 (CH_3); 23.8, 26.0 ($2CH_2$); 28.1, 28.14 ($2\underline{C}H_2CH_{alkene}$); 30.2, 30.3, 30.5, 30.6 ($4CH_2$); 30.7 ($2CH_2$); 30.77 ($2CH_2$); 30.8 ($2CH_2$); 33.1 (CH_2); 34.9 ($\underline{C}H_2CO$); 66.1 ($CO_2\underline{C}H_2$); 67.6 (d, *J* = 5.3, CH_2OP); 69.7 (d, *J* = 7.6, CH); 130.8, 130.9 ($2CH_{alkene}$); 175.4 (CO)

^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.16

$[\alpha]_D^{20}$: limited solubility

HRMS (MALDI, *m/z*): calculated for $C_{23}H_{45}NaO_7P$ ($[M+Na]^+$): 487.2801, found: 487.2798

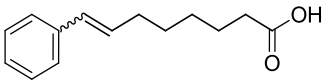
HPLC (method A, t_R, min): 12.35

4.2.5. Synthesis of final compounds **3a** and **2f**-**j**

4.2.5.1. Synthesis of carboxylic acids **44**, **45**, and **46**

(7*E,Z*)-8-Phenyloct-7-enoic acid, 42

Following the general procedure 4.2.1.15., alkene **42** was obtained from 7-bromoheptanoic acid (441 mg, 2.11 mmol) and benzaldehyde (103 µL, 1.00 mmol) in 61% yield. Chromatography: hexane to hexane/ethyl acetate, 1:1.

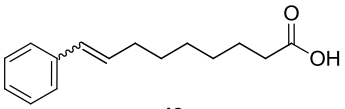


42

R_f: 0.61 (hexane/ethyl acetate, 8:2)
IR (ATR): 3024 (O-H); 1707 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture *E*:*Z* (2.3:1): δ 1.36-1.53 (m, 4H, $2CH_2$); 1.59-1.73 (m, 2H, CH_2); 2.23 (q, *J* = 6.8, 2H, $CH_2CH_{alkene(E)}$); 2.30-2.40 (m, 4H, $CH_2CH_{alkene(Z)}$, CH_2CO); 5.65 (dt, *J* = 11.7, 7.2, 1H, $CH_2CH_{alkene(Z)}$); 6.21 (dt, *J* = 15.8, 6.8, 1H, $CH_2CH_{alkene(E)}$); 6.39 (d, *J* = 15.6, 1H, $PhCH_{alkene(E)}$); 6.43 (d, *J* = 11.7, 1H, $PhCH_{alkene(Z)}$); 7.16-7.36 (m, 5H, $5CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture *E*:*Z* (2.3:1): δ 24.7 (CH_2); 28.5 ($CH_{2\ Z}$); 28.7 ($CH_{2\ E}$); 28.9 ($CH_{2\ Z}$); 29.1 ($CH_{2\ E}$); 32.9 (CH_2CH_{alkene}); 34.1 (CH_2CO); 126.1 ($2CH_{Ar\ E}$); 126.6 ($CH_{Ar\ Z}$); 127.0 ($CH_{Ar\ E}$); 128.3 ($2CH_{Ar\ Z}$); 128.6 ($2CH_{Ar\ E}$); 128.9 ($2CH_{Ar\ Z}$); 129.2 ($CH_{alkene(Z)}$); 130.2 ($CH_{alkene(E)}$); 130.8 ($CH_{alkene(E)}$); 132.8 ($CH_{alkene(Z)}$); 137.9 ($C_{Ar\ Z}$); 138.0 ($C_{Ar\ E}$); 179.9 (CO)
MS (ESI, *m/z*): 217.1 $[M-H]^-$

(8*E,Z*)-9-Phenylnon-8-enoic acid, 43

Following the general procedure 4.2.1.15., alkene **43** was obtained from 8-bromooctanoic acid (1g, 4.48 mmol) and benzaldehyde (0.36 mL, 3.58 mmol) in 70% yield. Chromatography: hexane to hexane/ethyl acetate, 1:1.



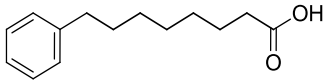
43

R_f: 0.52 (hexane/ethyl acetate, 8:2)
IR (ATR): 3024 (O-H); 1706 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture *E*:*Z* (1.5:1): δ 1.33-1.41 (m, 4H, $2CH_2$); 1.45-1.52 (m, 2H, CH_2); 1.59-1.72 (m, 2H, CH_2); 2.22 (q, *J* = 6.9, 2H, $CH_2CH_{alkene(E)}$); 2.37 (app q, *J* = 7.6, 4H, $CH_2CH_{alkene(Z)}$, CH_2CO); 5.66 (dt, *J* = 11.7, 7.3, 1H, $CH_2CH_{alkene(Z)}$); 6.23 (dt, *J* = 15.8, 6.8, 1H, $CH_2CH_{alkene(E)}$); 6.39 (d, *J* = 15.5, 1H, $PhCH_{alkene(E)}$); 6.44 (d, *J* = 11.5, 1H, $PhCH_{alkene(Z)}$); 7.17-7.37 (m, 5H, $5CH_{Ar}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture *E*:*Z* (1.5:1): δ 24.7 and 24.75 ($CH_{2\,E,Z}$); 28.6, 28.9, 29.0, 29.3 and 29.9 ($3CH_{2\,Z,E}$); 33.1 ($\underline{C}H_2CH_{alkene}$); 34.1 and 34.14 ($\underline{C}H_2CO_{\,E,Z}$); 126.0 ($2CH_{Ar\,E}$); 126.6 ($CH_{Ar\,Z}$); 126.9 ($CH_{Ar\,E}$); 128.2 ($2CH_{Ar\,Z}$); 128.6 ($2CH_{Ar\,E}$); 128.9 ($2CH_{Ar\,Z}$); 129.0 ($CH_{alkene(Z)}$); 130.0 ($CH_{alkene(E)}$); 131.06 ($CH_{alkene(E)}$); 133.10 ($CH_{alkene(Z)}$); 137.9 ($C_{Ar\,Z}$); 138.0 ($C_{Ar\,E}$); 180.1 (CO)
MS (ESI, *m/z*): 231.1 $[M-H]^-$

8-Phenyloctanoic acid, 44

Following the general procedure 4.2.1.1., carboxylic acid **44** was obtained from alkene **42** (60 mg, 0.27 mmol) at room temperature in 97% yield. The spectroscopic data correspond with those previously reported.[106]

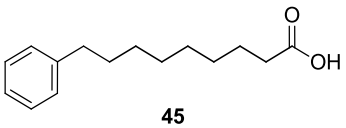


44

R_f: 0.60 (hexane/ethyl acetate, 8:2)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.41 (app s, 6H, $3CH_2$); 1.70 (br s, 4H, $2CH_2$); 2.42 (t, *J* = 7.5, 2H, CH_2CO); 2.67 (t, *J* = 7.7, 2H, $PhC\underline{H}_2$); 7.23-7.26 (m, 3H, $3CH_{Ar}$); 7.32-7.37 (m, 2H, $2CH_{Ar}$)

9-Phenylnonanoic acid, 45

Following the general procedure 4.2.1.1., carboxylic acid **45** was obtained from alkene **43** (300 mg, 1.29 mmol) at room temperature in 81% yield.



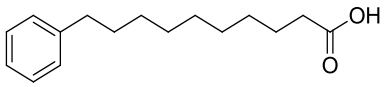
45

R_f: 0.61 (hexane/ethyl acetate, 8:2)
IR (ATR): 3063 (O-H); 1707 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.32 (app s, 8H, $4CH_2$); 1.56-1.65 (m, 4H, $2CH_2$); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 2.60 (t, *J* = 7.7, 2H, $PhC\underline{H}_2$); 7.16-7.19 (m, 3H, $3CH_{Ar}$); 7.25-7.30 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 24.8, 29.2, 29.3, 29.35, 29.4, 31.6 ($6CH_2$); 34.2 ($\underline{C}H_2CO$); 36.1 ($Ph\underline{C}H_2$); 125.7 (CH_{Ar}); 128.4 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.0 (C_{Ar}); 180.5 (CO)
MS (ESI, *m/z*): 233.1 $[M-H]^-$

10-Phenyldecanoic acid, 46

To a stirred solution of 10-phenyldecanol (500 mg, 2.10 mmol, 1 equiv) in dry DMF (2 mL), pyridinium dichromate (2.92 g, 3.89 mmol, 3.7 equiv) was added and the reaction was stirred at room temperature overnight. Then, solvent was evaporated

under reduced pressure and the crude was purified by flash chromatography (dichloromethane to dichloromethane/methanol, 10:1) affording carboxylic acid **46** in 52% yield.



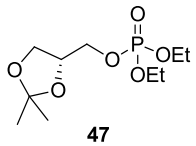
46

R_f: 0.6 (dichloromethane/methanol, 9:1)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.32 (m, 10H, $5CH_2$); 1.63-1.65 (m, 4H, $2CH_2$); 2.34 (t, *J* = 7.4, 2H, CH_2CO); 2.60 (t, *J* = 7.5, 2H, $PhCH_2$); 7.18-7.22 (m, 3H, $3CH_{Ar}$); 7.27-7.33 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 24.8, 29.2, 29.4, 29.41, 29.5, 29.6, 31.6 ($7CH_2$); 34.2 (CH_2CO); 36.1 ($PhCH_2$); 125.7 (CH_{Ar}); 128.4 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.0 (C_{Ar}); 180.3 (CO)

4.2.5.2. Synthesis of the phosphorylated diols **48** and **51**

[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]methyl diethyl phosphate, 47

To a solution of (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (0.3 mL, 2.43 mmol, 1 equiv) in anhydrous dichloromethane (8 mL), diethyl chlorophosphate (0.44 mL, 3.04 mmol, 1.25 equiv) and potassium *tert*-butoxide (408 mg, 3.64 mmol, 1.5 equiv) were added and the mixture was stirred at room temperature for 48 h. Then, the reaction was quenched with a saturated aqueous solution of NH_4Cl (12 mL) and stirred for 10 additional min. The mixture was extracted with dichloromethane and the organic phase was dried over Na_2SO4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (dichloromethane/ethyl acetate, 10:1 to 1:1), to afford pure product **47** in 92% yield.

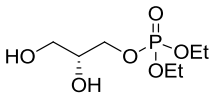


47

R_f: 0.26 (dichloromethane/ethyl acetate, 8:2)
IR (ATR): 1021 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.31 (td, *J* = 7.1, 0.9, 6H, $2CH_2CH_3$); 1.33 (s, 3H, $CH_{3\ acetal}$); 1.39 (s, 3H, $CH_{3\ acetal}$); 3.81 (dd, *J* = 8.6, 5.5, 1H, ½CH_2O); 3.92-4.05 (m, 3H, ½CH_2O, CH_2OP); 4.07-4.16 (m, 4H, $2CH_2CH_3$): 4.24-4.33 (m, 1H, CH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 16.2 (d, *J* = 6.7, $2CH_2CH_3$); 25.4 ($CH_{3\ acetal}$); 26.8 ($CH_{3\ acetal}$); 64.1 (d, *J* = 5.7, $2CH_2CH_3$); 66.3 (CH_2O); 67.4 (d, *J* = 5.8, CH_2OP); 74.2 (d, *J* = 8.2, CH); 109.9 (C)

(2*R*)-2,3-Dihydroxypropyl diethyl phosphate, 48

Following the general procedure 4.2.1.6., diol **48** was obtained from **47** (600 mg, 2.24 mmol) in 50% yield. Chromatography: dichloromethane/methanol, 50:1 to 10:1.

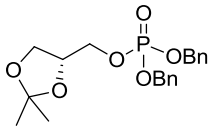


48

R_f: 0.11 (dichloromethane/methanol, 10:1)
IR (ATR): 3405 (O-H); 1256 (P=O); 1027 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.34 (t, *J* = 7.1, 6H, $2CH_3$); 3.21 (br s, 2H, 2OH); 3.60-3.73 (m, 2H, CH_2OH); 3.75-3.83 (m, 1H, ½CH_2OP); 3.86-3.94 (m, 1H, CH); 4.06-4.18 (m, 5H, ½CH_2OP, $2CH_2CH_3$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 16.2 (d, *J* = 6.7, $2CH_3$); 62.9 (CH_2OH); 64.5 (d, *J* = 6.0, $2CH_2CH_3$); 68.5 (d, *J* = 5.8, CH_2OP); 70.8 (d, *J* = 5.5, CH)
$[\alpha]_D^{20}$: -6.5 (c = 1.11, methanol)

Dibenzyl [(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methyl phosphate, 50

Following the general procedure 4.2.1.13., compound **50** was obtained from (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (0.2 mL, 1.62 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.97 mL, 2.91 mmol) in 76% yield. Chromatography: hexane to hexane/ethyl acetate, 1:1.

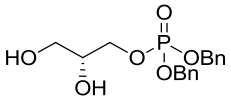


50

R_f: 0.4 (hexane/ethyl acetate, 1:1)
IR (ATR): 1255 (P=O); 991 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.32 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); 3.72 (dd, *J* = 8.6, 5.5, 1H, ½CH_2O); 3.87-4.04 (m, 3H, ½CH_2O, CH_2OP); 4.20 (qt, *J* = 5.7, 1H, CH); 4.97-5.13 (m, 4H, $2PhCH_2$); 7.34 (app s, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.3, 26.8 ($2CH_3$); 66.2 (CH_2O); 67.5 (d, *J* = 5.9, CH_2OP); 69.5 (d, *J* = 5.6, $2PhCH_2$); 74.0 (d, *J* = 8.3, CH); 109.9 (C); 128.1 ($4CH_{Ar}$); 128.7 ($6CH_{Ar}$); 135.8 (d, *J* = 6.6, $2C_{Ar}$)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 2.03
$[\alpha]_D^{20}$: +1.6 (c = 1.00, methanol)
MS (ESI, *m/z*): 393.1 $[M+H]^+$

Dibenzyl (2*R*)-2,3-dihydroxypropyl phosphate, 51

Following the general procedure 4.2.1.6., diol **51** was obtained from **50** (390 mg, 0.99 mmol) in 65% yield. Chromatography: ethyl acetate to ethyl acetate/ethanol, 7:3.



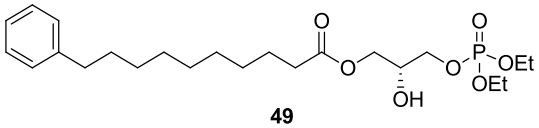
51

R_f: 0.56 (ethyl acetate)
IR (ATR): 3368 (O-H); 1255 (P=O); 1013 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.53 (br s, 2H, OH); 3.52-3.65 (m, 2H, CH_2OH); 3.78-3.85 (m, 1H, CH); 4.03 (dd, *J* = 9.5, 5.0, 2H, CH_2OP); 4.98-5.11 (m, 4H, $2PhCH_2$); 7.35 (app s, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 62.7 (CH_2OH); 68.8 (d, *J* = 5.9, CH_2OP); 69.9 (d, *J* = 5.8, $2PhCH_2$); 70.7 (d, *J* = 5.2, CH); 128.2 ($4CH_{Ar}$); 128.8 ($4CH_{Ar}$); 128.9 ($2CH_{Ar}$); 135.60 (d, *J* = 6.6, $2C_{Ar}$)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.19
$[\alpha]_D^{20}$: -4.6 (c = 0.99, methanol)
MS (ESI, *m/z*): 353.0 $[M+H]^+$

4.2.5.3. Synthesis of esters **49** and **52**-**56**

(2*R*)-3-[(Diethoxyphosphoryl)oxy]-2-hydroxypropyl 10-phenyldecanoate, 49

Following the general procedure 4.2.1.14., ester **49** was obtained from carboxylic acid **46** (136 mg, 0.55 mmol) and diol **48** (250 mg, 1.10 mmol) in 30% yield. Chromatography: dichloromethane/methanol, 200:1 to 50:1.

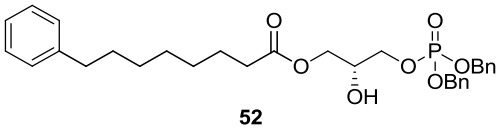


49

R_f: 0.45 (dichloromethane/methanol, 10:1)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.28 (app s, 10H, $5CH_2$); 1.35 (td, *J* = 7.1, 0.9, 6H, $2CH_3$); 1.55-1.62 (m, 4H, $2CH_2$); 2.33 (t, *J* = 7.6, 2H, CH_2CO); 2.59 (t, *J* = 7.7, 2H, $PhCH_2$); 4.02-4.26 (m, 9H, CH, $2CH_2CH_3$, CH_2OP, CO_2CH_2); 7.14-7.18 (m, 3H, $3CH_{Ar}$); 7.24-7.30 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 16.2 (d, *J* = 6.6, $2CH_3$); 25.0, 29.2, 29.4, 29.41, 29.5, 29.6, 31.6 ($7CH_2$); 34.2 (CH_2CO); 36.1 ($PhCH_2$); 64.44 (d, *J* = 5.4, CH_2CH_3); 64.45 (d, *J* = 5.4, CH_2CH_3); 64.5 (CO_2CH_2); 68.8 (d, *J* = 5.7, CH_2OP); 69.0 (d, *J* = 5.5, CH); 125.7 (CH_{Ar}); 128.3 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.0 (C_{Ar}); 174.0 (CO)
MS (ESI, *m/z*): 459.2 $[M+H]^+$

(2*R*)-3-{[Bis(benzyloxy)phosphoryl]oxy}-2-hydroxypropyl 8-phenyloctanoate, 52

Following the general procedure 4.2.1.14., ester **52** was obtained from carboxylic acid **44** (37 mg, 0.17 mmol) and diol **51** (120 mg, 0.34 mmol) in 53% yield. Chromatography: dichloromethane to dichloromethane/methanol, 95:5.

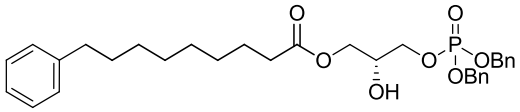


52

R_f: 0.44 (dichloromethane/methanol, 95:5)
IR (ATR): 3382 (O-H); 1738 (C=O); 1265 (P=O); 1016 (P-O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.22-1.35 (m, 6H, 3CH_2); 1.48-1.64 (m, 4H, 2CH_2); 2.19-2.32 (m, 2H, CH_2CO); 2.52-2.59 (m, 2H, PhCH_2); 3.42-4.70 (m, 1H, ½CH_2OP); 3.91-4.36 (m, 4H, CH, ½CH_2OP, CO_2CH_2); 5.01-5.10 (m, 4H, 2OCH_2Ph); 7.09-7.14 (m, 3H, 3CH_{Ar}); 7.20-7.25 (m, 2H, 2CH_{Ar}); 7.30-7.39 (m, 10H, 10CH_{Ar})
^{13}C-NMR (methanol-d_4, 75 MHz): δ 26.0, 30.07, 30.1, 30.2, 32.6 (5CH_2); 34.7 (CH_2CO); 36.9 (PhCH_2); 65.4 (CO_2CH_2); 68.9 (d, *J* = 7.9, CH); 69.5 (d, *J* = 6.1, CH_2OP); 70.9 (d, *J* = 5.9, 2OCH_2Ph); 126.6 (CH_{Ar}); 129.2 (2CH_{Ar}); 129.24 (2CH_{Ar}); 129.4 (4CH_{Ar}); 129.7 (4CH_{Ar}); 129.8 (2CH_{Ar}); 137.1 (d, *J* = 6.4, 2C_{Ar}); 143.9 (C_{Ar}); 175.1 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 1.54
$[\alpha]_D^{20}$: +1.6 (c = 1.19, methanol)
MS (ESI, *m/z*): 537.2 $[M-H_2O+H]^+$; 555.2 $[M+H]^+$

(2*R*)-3-{[Bis(benzyloxy)phosphoryl]oxy}-2-hydroxypropyl 9-phenylnonanoate, 53

Following the general procedure 4.2.1.14., ester **53** was obtained from carboxylic acid **45** (90 mg, 0.38 mmol) and diol **51** (271 mg, 0.76 mmol) in 58% yield. Chromatography: dichloromethane to dichloromethane/methanol, 95:5.



53

R_f: 0.44 (dichloromethane/methanol, 95:5)
IR (ATR): 3325 (O-H); 1736 (C=O); 1245 (P=O); 1013 (P-O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.25-1.35 (m, 8H, 4CH_2); 1.55-1.64 (m, 4H, 2CH_2); 2.30 (t, *J* = 7.4, 2H, CH_2CO); 2.58 (t, *J* = 7.7, 2H, PhCH_2); 3.91-3.98 (m, 1H, CH); 3.98-4.03 (m, 2H, CH_2OP); 4.07 (dd, *J* = 5.1, 1.5, 2H, CO_2CH_2); 5.05 (s, 2H,

OC$\underline{H}_2$Ph); 5.08 (s, 2H, OC$\underline{H}_2$Ph); 7.10-7.16 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar}); 7.36 (app s, 10H, 10CH_{Ar})

^{13}C-NMR (methanol-d_4, 75 MHz): δ 26.8, 30.1, 30.2, 30.3, 30.4, 32.7 (6CH_2); 34.9 ($\underline{C}H_2$CO); 36.9 (Ph$\underline{C}H_2$); 65.4 ($CO_2\underline{C}H_2$); 68.9 (d, *J* = 8.0, CH); 69.6 (d, *J* = 6.4, CH_2OP); 70.9 (d, *J* = 5.8, 2O$\underline{C}H_2$Ph); 126.6 (CH_{Ar}); 129.20 (4CH_{Ar}); 129.24 (2CH_{Ar}); 129.4 (2CH_{Ar}); 129.7 (4CH_{Ar}); 129.8 (2CH_{Ar}); 137.2 (d, *J* = 6.5, 2C_{Ar}); 144.0 (C_{Ar}); 175.2 (CO)

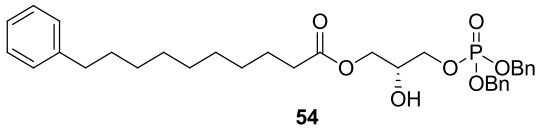
^{31}P-NMR (methanol-d_4, 121 MHz): δ 1.61

$[\alpha]_D^{20}$: +0.02 (c = 0.65, methanol)

MS (ESI, *m/z*): 551.2 [M-H_2O+H]$^+$; 569.3 [M+H]$^+$

(2*R*)-3-{[Bis(benzyloxy)phosphoryl]oxy}-2-hydroxypropyl 10-phenyl decanoate, 54

Following the general procedure 4.2.1.14., ester **54** was obtained from carboxylic acid **46** (82 mg, 0.33 mmol) and diol **51** (233 mg, 0.66 mmol) in 16% yield. Chromatography: dichloromethane/ethyl acetate, 100:1 to 1:1.



54

R_f: 0.43 (dichloromethane/ethyl acetate, 8:2)

IR (ATR): 1738 (C=O); 1022 (P-O)

^{1}H-NMR (C_6D_6, 300 MHz): δ 1.15-1.20 (m, 10H, 5CH_2); 1.49-1.58 (m, 4H, 2CH_2); 2.11 (t, *J* = 7.5, 2H, CH_2CO); 2.51 (t, *J* = 7.5, 2H, PhC$\underline{H}_2$); 3.95-4.06 (m, 3H, CH, CH_2OP); 4.12-4.24 (m, 2H, CO_2CH_2); 4.86-5.00 (m, 4H, 2OC$\underline{H}_2$Ph); 7.03-7.12 (m, 9H, 9CH_{Ar}); 7.16-7.22 (m, 6H, 6CH_{Ar})

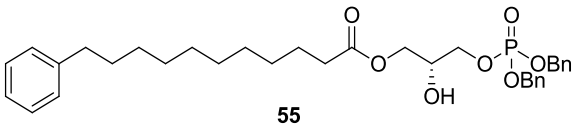
^{13}C-NMR (C_6D_6, 75 MHz): δ 25.2, 29.4, 29.6, 29.7, 29.8, 29.9, 31.9 (7CH_2); 34.2 ($\underline{C}H_2$CO); 36.4 (Ph$\underline{C}H_2$); 64.6 ($CO_2\underline{C}H_2$); 69.0 (d, *J* = 5.4, CH); 69.4 (d, *J* = 5.9, CH_2OP); 69.7 (d, *J* = 5.0, O$\underline{C}H_2$Ph); 69.8 (d, *J* = 5.1, O$\underline{C}H_2$Ph); 126.0 (CH_{Ar}); 128.5 (2CH_{Ar}); 128.6 (2CH_{Ar}); 128.8 (2CH_{Ar}); 128.83 (2CH_{Ar}); 128.9 (6CH_{Ar}); 136.4 (d, *J* = 6.7, 2C_{Ar}), 143.0 (C_{Ar}); 173.2 (CO)

^{31}P-NMR (C_6D_6, 121 MHz): δ 3.92

MS (ESI, *m/z*): 565.2 [M-H_2O+H]$^+$; 583.3 [M+H]$^+$

(2*R*)-3-{[Bis(benzyloxy)phosphoryl]oxy}-2-hydroxypropyl 11-phenyl undecanoate, 55

Following the general procedure 4.2.1.14., ester **55** was obtained from 11-phenylundecanoic acid (37 mg, 0.14 mmol) and diol **51** (99 mg, 0.28 mmol) in 18% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.



55

R_f: 0.28 (hexane/ethyl acetate, 6:4)

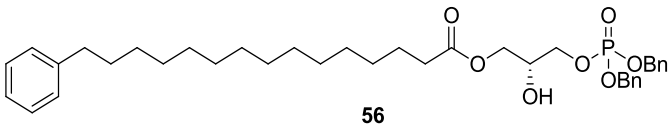
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.19-1.36 (m, 12H, $6CH_2$); 1.49-1.65 (m, 4H, $2CH_2$); 2.19-2.37 (m, 2H, CH_2CO); 2.58 (t, *J* = 7.6, 2H, $PhCH_2$); 3.62-4.18 (m, 5H, CH, CH_2OP, CO_2CH_2); 4.97-5.12 (m, 4H, $2OCH_2Ph$); 7.10-7.16 (m, 3H, $3CH_{Ar}$); 7.21-7.26 (m, H, $2CH_{Ar}$); 7.28-7.41 (app s, 10H, 10 CH_{Ar})

^{13}C-NMR (methanol-d_4, 75 MHz): δ 25.9, 30.2, 30.28, 30.34 ($4CH_2$); 30.5 ($2CH_2$); 30.6, 32.8 ($2CH_2$); 34.8 (CH_2CO); 36.9 ($PhCH_2$); 65.4 (CO_2CH_2); 68.9 (d, *J* = 8.0, CH); 69.5 (d, *J* = 6.0, CH_2OP); 70.9 (d, *J* = 5.9, $2OCH_2Ph$); 126.6 (CH_{Ar}); 129.2 ($2CH_{Ar}$); 129.23 ($4CH_{Ar}$); 129.4 ($4CH_{Ar}$); 129.7 ($2CH_{Ar}$); 129.8 ($2CH_{Ar}$); 137.1 (d, *J* = 6.1, $2C_{Ar}$), 144.0 (C_{Ar}); 175.1 (CO)

^{31}P-NMR (methanol-d_4, 121 MHz): δ 1.61

(2*R*)-3-{[Bis(benzyloxy)phosphoryl]oxy}-2-hydroxypropyl 15-phenyl pentadecanoate, 56

Following the general procedure 4.2.1.14., ester **56** was obtained from 15-phenylpentadecanoic acid (80 mg, 0.25 mmol) and diol **51** (177 mg, 0.50 mmol) in 18% yield. Chromatography: dichloromethane/ethyl acetate, 100:1 to 1:1.



56

R_f: 0.55 (dichloromethane/ethyl acetate, 8:2)

IR (ATR): 3334 (O-H); 1740 (C=O); 1259 (P=O); 1017 (P-O)

^{1}H-NMR (C_6D_6, 500 MHz): δ 1.20-1.36 (m, 20H, $10CH_2$); 1.54-1.58 (m, 4H, $2CH_2$); 2.10 (t, *J* = 7.5, 2H, CH_2CO); 2.52 (t, *J* = 7.5, 2H, $PhCH_2$); 3.84-3.89 (m, 1H, CH); 3.91-3.99 (m, 2H, CH_2OP); 4.07 (dd, *J* = 11.4, 5.3, 1H, ½CO_2CH_2); 4.11 (dd, *J* = 11.4, 5.7, 1H, ½CO_2CH_2); 4.85-4.96 (m, 4H, $2OCH_2Ph$); 7.04-7.12 (m, 8H, $8CH_{Ar}$); 7.15-7.22 (m, 7H, $7CH_{Ar}$)

^{13}C-NMR (C_6D_6, 125 MHz): δ 25.3, 29.5, 29.7, 29.72, 29.9, 30.0, 30.08, 30.1, 30.14 ($9CH_2$); 30.2 ($2CH_2$); 32.0 (CH_2); 34.2 (CH_2CO); 36.4 ($PhCH_2$); 64.5 (CO_2CH_2); 69.2 (d, *J* = 4.8, CH); 69.4 (d, *J* = 5.6, CH_2OP); 69.7 (d, *J* = 5.5, OCH_2Ph); 69.74 (d, *J* = 5.5, OCH_2Ph); 126.2 (CH_{Ar}); 128.5 ($4CH_{Ar}$); 128.54 ($2CH_{Ar}$); 128.8 ($2CH_{Ar}$); 128.84 ($2CH_{Ar}$); 128.9 ($4CH_{Ar}$); 136.4 (d, *J* = 6.5, $2C_{Ar}$); 143.1 (C_{Ar}); 173.1 (CO)

^{31}P-NMR (C_6D_6, 121 MHz): δ 4.60

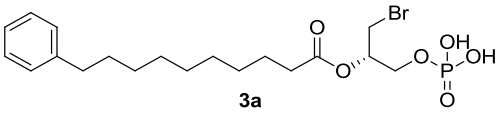
MS (ESI, *m/z*): 653.4 $[M+H]^+$

4.2.5.4. Synthesis of final compounds **3a** and **2f-j**

(1*S*)-2-Bromo-1-[(phosphonooxy)methyl]ethyl 10-phenyldecanoate, 3a

Thoroughly dried diethyl phosphate **49** (76 mg, 0.17 mmol, 1 equiv) was dissolved in anhydrous dichloromethane (2 mL) and TMSBr (0.22 mL, 1.66 mmol, 10 equiv) was added dropwise at room temperature. The reaction mixture was stirred for 4 h at room temperature, after which the solvent was evaporated at reduced pressure and the crude was dissolved in 95% methanol in water (5 mL) and stirred for an additional hour. Evaporation of the solvent afforded compound **3a** in 90% yield.

The specific rotation value agrees with that obtained for compound **(*S*)-3a**, confirming the *S* configuration of the chiral center.

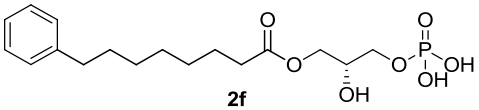


3a

IR (ATR): 3360 (O-H); 1738 (C=O), 1199 (P=O); 1025 (P-OH)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 1.32 (m, 10H, 5CH_2); 1.60-1.65 (m, 4H, 2CH_2); 2.37 (t, *J* = 7.3, 2H, CH_2CO); 2.59 (t, *J* = 7.6, 2H, PhCH_2); 3.58 (dd, *J* = 10.9, 5.9, 1H, ½CH_2Br); 3.66 (dd, *J* = 10.9, 4.9, 1H, ½CH_2Br); 4.12-4.18 (m, 2H, CH_2OP); 5.14-5.19 (m, 1H, CH); 7.11-7.17 (m, 3H, 3CH_{Ar}); 7.23 (t, *J* = 7.5, 2H, 2CH_{Ar})
^{13}C-NMR (methanol-d_4, 125 MHz): δ 26.0, 30.1, 30.3, 30.31 (4CH_2); 30.5 (2CH_2); 30.8 (CH_2Br); 32.7 (CH_2); 34.9 (CH_2CO); 36.9 (PhCH_2); 66.3 (d, *J* = 5.0, CH_2OP); 72.5 (d, *J* = 8.3, CH); 126.6 (CH_{Ar}); 129.2 (2CH_{Ar}); 129.4 (2CH_{Ar}); 144.0 (C_{Ar}); 174.3 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.10
$[\alpha]_D^{20}$: +4.9 (c = 0.50, methanol)
HRMS (ESI, *m/z*): calculated for $C_{19}H_{29}{}^{79}BrO_6P$ ([M(^{79}Br)-H]$^-$: 463.0891, found: 463.0902; calculated for $C_{19}H_{29}{}^{81}BrO_6P$ ([M(^{81}Br)-H]$^-$: 465.0870, found: 465.0880
HPLC (method B, t_R, min): 26.05

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl 8-phenyloctanoate, 2f

Following the general procedure 4.2.1.1., compound **2f** was obtained from dibenzyl phosphate **52** (63 mg, 0.11 mmol) at room temperature in 80% yield.



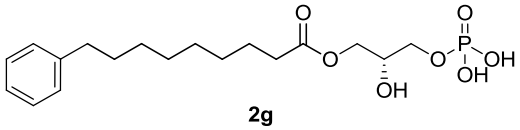
2f

IR (ATR): 1737 (C=O); 1027 (P-O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.33 (app s, 6H, 3CH_2); 1.58-1.60 (m, 4H, 2CH_2); 2.27-2.37 (m, 2H, CH_2CO); 2.59 (t, *J* = 7.5, 2H, PhCH_2); 3.59-3.61 (m, 3H, CH, CH_2OP); 3.99-4.41 (m, 2H, CO_2CH_2); 7.10-7.16 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar})

^{13}C-NMR (methanol-d_4, 75 MHz): δ 25.9, 30.07, 30.1, 30.2, 32.6 (5CH_2); 34.8 ($\underline{C}H_2CO$); 36.9 (Ph$\underline{C}H_2$); 65.8 ($CO_2\underline{C}H_2$); 68.1 (d, *J* = 5.0, CH_2OP); 69.3 (br s, CH); 126.6 (CH_{Ar}); 129.2 (2CH_{Ar}); 129.4 (2CH_{Ar}); 143.9 (C_{Ar}); 175.3 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.04
$[\alpha]_D^{20}$: +2.8 (c = 1.58, methanol)
HRMS (ESI, *m/z*): calculated for $C_{17}H_{26}O_7P$ ($[M-H]^-$): 373.1422, found: 373.1432
HPLC (method A, t_R, min): 8.07

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl 9-phenylnonanoate, 2g

Following the general procedure 4.2.1.1., compound **2g** was obtained from dibenzyl phosphate **53** (90 mg, 0.16 mmol) at room temperature in 99% yield.

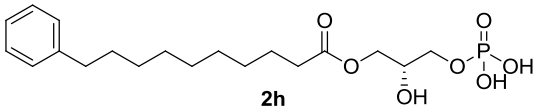


2g

IR (ATR): 3355 (O-H); 1737 (C=O); 1259 (P=O); 1029 (P-O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.32 (app s, 8H, 4CH_2); 1.60 (br s, 4H, 2CH_2); 2.34 (t, *J* = 7.4, 2H, C$\underline{H}_2$CO); 2.59 (t, *J* = 7.8, 2H, Ph$\underline{C}H_2$); 3.67-3.79 (m, 1H, CH); 3.98-4.00 (m, 2H, CH_2OP); 4.07-4.21 (m, 2H, CO_2CH_2); 7.09-7.17 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar})
^{13}C-NMR (methanol-d_4, 75 MHz): δ 25.9, 30.1, 30.2, 30.3, 30.4, 32.7 (6CH_2); 34.8 ($\underline{C}H_2CO$); 36.9 (Ph$\underline{C}H_2$); 65.8 ($CO_2\underline{C}H_2$); 68.1 (d, *J* = 6.0, CH_2OP); 69.3 (d, *J* = 8.3, CH); 126.6 (CH_{Ar}); 129.2 (2CH_{Ar}); 129.4 (2CH_{Ar}); 143.9 (C_{Ar}); 175.3 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.05
$[\alpha]_D^{20}$: +2.5 (c = 1.13, methanol)
HRMS (ESI, *m/z*): calculated for $C_{18}H_{28}O_7P$ ($[M-H]^-$): 387.1578, found: 387.1591
HPLC (method A, t_R, min): 8.34

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl 10-phenyldecanoate, 2h

Following the general procedure 4.2.1.1., compound **2h** was obtained from dibenzyl phosphate **54** (20 mg, 34 µmol) at room temperature in 80% yield.



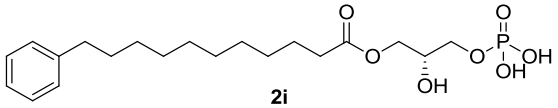
2h

IR (ATR): 1739 (C=O); 1051 (P-OH)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 1.29-1.32 (m, 10H, 5CH_2); 1.61 (m, 4H, 2CH_2); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 2.59 (t, *J* = 7.6, 2H, PhC$\underline{H}_2$); 3.96-4.01 (m, 3H, CH, CH_2OP); 4.11 (dd, *J* = 11.3, 5.3, 1H, ½CO_2CH_2); 4.17 (dd, *J* = 11.3, 4.1, 1H, ½CO_2CH_2); 7.11-7.16 (m, 3H, 3CH_{Ar}); 7.23 (t, *J* = 7.5, 2H, 2CH_{Ar})

^{13}C-NMR (methanol-d_4, 125 MHz): δ 26.0, 30.2, 30.3, 30.34 ($4CH_2$); 30.5 ($2CH_2$); 32.7 (CH_2); 34.9 ($\underline{C}H_2CO$); 36.9 ($Ph\underline{C}H_2$); 65.9 ($CO_2\underline{C}H_2$); 68.1 (d, *J* = 5.7, CH_2OP); 69.4 (d, *J* = 8.1, CH); 126.6 (CH_{Ar}); 129.2 ($2CH_{Ar}$); 129.4 ($2CH_{Ar}$); 144.0 (C_{Ar}); 175.4 (CO)
^{31}P-NMR (methanol-d_4, 202 MHz): δ 3.14
$[\alpha]_D^{20}$: +2.3 (c = 0.40, methanol)
HRMS (ESI, *m/z*): calculated for $C_{19}H_{30}O_7P$ ($[M-H]^-$): 401.1735, found: 401.1734
HPLC (method B, t_R, min): 24.37

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl 11-phenylundecanoate, 2i

Following the general procedure 4.2.1.1., compound **2i** was obtained from dibenzyl phosphate **55** (5 mg, 8 µmol) at room temperature in 82% yield.

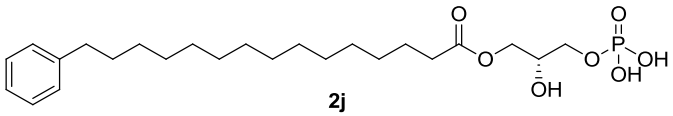


2i

IR (ATR): 3387 (O-H); 1739 (C=O); 1262 (P=O); 1014 (P-OH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.26-1.32 (m, 12H, $6CH_2$); 1.53-1.68 (m, 4H, $2CH_2$); 2.35 (t, *J* = 7.5, 2H, CH_2CO); 2.59 (t, *J* = 7.7, 2H, $PhC\underline{H}_2$); 3.69-3.74 (m, 1H, CH); 3.98-4.02 (m, 2H, CH_2OP); 4.08-4.16 (m, 2H, CO_2CH_2); 7.10-7.16 (m, 3H, $3CH_{Ar}$); 7.21-7.26 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 25.8, 30.2, 30.3, 30.4, 30.55, 30.56, 30.7, 32.8 ($8CH_2$); 34.9 ($\underline{C}H_2CO$); 36.9 ($Ph\underline{C}H_2$); 65.8 ($CO_2\underline{C}H_2$); 68.1 (d, *J* = 5.6, CH_2OP); 69.3 (d, *J* = 8.9, CH); 126.6 (CH_{Ar}); 129.2 ($2CH_{Ar}$); 129.4 ($2CH_{Ar}$); 144.0 (C_{Ar}); 175.3 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.07
$[\alpha]_D^{20}$: +0.2 (c = 0.32, methanol)
HRMS (ESI, *m/z*): calculated for $C_{20}H_{32}O_7P$ ($[M-H]^-$): 415.1891, found: 415.1906

(2*R*)-2-Hydroxy-3-(phosphonooxy)propyl 15-phenylpentadecanoate, 2j

Following the general procedure 4.2.1.1., compound **2j** was obtained from dibenzyl phosphate **56** (20 mg, 31 µmol) at room temperature in 90% yield.



2j

IR (ATR): 1738 (C=O); 1259 (P=O); 1083 (P-OH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.28-1.32 (m, 20H, $10CH_2$); 1.57-1.64 (m, 4H, $2CH_2$); 2.35 (t, *J* = 7.4, 2H, CH_2CO); 2.59 (t, *J* = 7.5, 2H, $PhC\underline{H}_2$); 3.97-4.35 (m, 5H, CO_2CH_2, CH, CH_2OP); 7.08-7.16 (m, 3H, $3CH_{Ar}$); 7.21-7.26 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 26.0, 30.2, 30.3, 30.4, 30.59, 30.6, 30.7 ($7CH_2$); 30.73 ($2CH_2$); 30.75 ($2CH_2$); 32.8 (CH_2); 34.9 ($\underline{C}H_2CO$); 36.9 ($Ph\underline{C}H_2$); 64.9 ($CO_2\underline{C}H_2$);

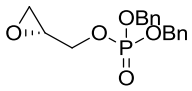
67.1 (d, *J* = 5.3, CH_2OP); 68.3 (d, *J* = 8.3, CH); 126.6 (CH_{Ar}); 129.2 ($2CH_{Ar}$); 129.4 ($2CH_{Ar}$); 144.0 (C_{Ar}); 175.4 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 3.11
$[\alpha]_D^{20}$: +4.2 (c = 0.40, methanol)
HRMS (ESI, *m/z*): calculated for $C_{24}H_{40}O_7P$ ($[M-H]^-$): 471.2517, found: 471.2520
HPLC (method B, t_R, min): 33.42

4.2.6. Synthesis of final compounds **(*S*)-** and **(*R*)-3a**

4.2.6.1. Synthesis of bromoalcohols **(*S*)-** and **(*R*)-58**

Dibenzyl (2*R*)-oxiran-2-ylmethyl phosphate, (*R*)-57

Following the general procedure 4.2.1.13., compound **(*R*)-57** was obtained from (2*S*)-oxiran-2-ylmethanol (0.3 mL, 4.52 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (3.04 mL, 9.04 mmol) in 76% yield. Chromatography: dichloromethane to dichloromethane/ethyl acetate, 20:1.

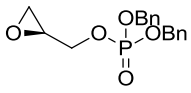


(*R*)-57

R_f: 0.35 (hexane/ethyl acetate, 1:1)
IR (ATR): 1275 (P=O); 1013 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.59 (dd, *J* = 4.8, 2.6, 1H, ½$CH_{2\ epox}$); 2.78 (t, *J* = 4.7, 1H, ½$CH_{2\ epox}$); 3.04-3.25 (m, 1H, CH); 3.89 (ddd, *J* = 11.7, 8.5, 5.9, 1H, ½CH_2OP); 4.12-4.27 (m, 1H, ½CH_2OP); 4.97-5.15 (m, 4H, 2PhCH_2); 7.35 (app s, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 44.7 ($CH_{2\ epox}$); 50.0 (d, *J* = 8.0, CH); 68.1 (d, *J* = 5.5, CH_2OP); 69.6 (d, *J* = 5.3, PhCH_2); 69.61 (d, *J* = 5.5, PhCH_2); 128.1 ($4CH_{Ar}$); 128.7 ($6CH_{Ar}$); 135.8 (d, *J* = 6.8, $2C_{Ar}$)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 2.03
$[\alpha]_D^{20}$: -8.8 (c = 1.00, methanol)
MS (ESI, *m/z*): 335.9 $[M+H]^+$

Dibenzyl (2*S*)-oxiran-2-ylmethyl phosphate, (*S*)-57

Following the general procedure 4.2.1.13., compound **(*S*)-57** was obtained from (2*R*)-oxiran-2-ylmethanol (250 mg, 3.37 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (2.33 g, 6.75 mmol) in 95% yield. Chromatography: dichloromethane to dichloromethane/ethyl acetate, 9:1. The spectroscopic data are in agreement with those reported for **(*R*)-57**.

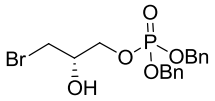


(*S*)-57

$[\alpha]_D^{20}$: +1.2 (c = 1.20, methanol)

Dibenzyl (2*S*)-3-bromo-2-hydroxypropyl phosphate, (*S*)-58

Following the general procedure 4.2.1.16., bromoalcohol **(*S*)-58** was obtained from oxirane **(*R*)-57** (990 mg, 2.96 mmol) in 89% yield.

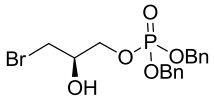


(*S*)-58

R_f: 0.41 (dichloromethane/ethyl acetate, 10:1)
IR (ATR): 3371 (O-H); 1009 (P=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.96 (br s, 1H, OH); 3.36 (d, *J* = 5.7, 2H, CH_2Br); 3.88-3.95 (m, 1H, CH); 4.06-4.12 (m, 2H, CH_2OP); 5.00-5.12 (m, 4H, 2PhCH_2); 7.36 (app s, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 33.3 (CH_2Br); 69.2 (d, *J* = 6.0, CH_2OP); 69.7 (d, *J* = 6.6, CH); 70.3 (d, *J* = 5.8, PhCH_2); 70.4 (d, *J* = 5.6, PhCH_2); 128.3 ($4CH_{Ar}$); 128.9 ($4CH_{Ar}$); 129.1 ($2CH_{Ar}$); 135.3 (d, *J* = 6.3, $2C_{Ar}$)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 2.48
$[\alpha]_D^{20}$: -2.1 (c = 1.44, methanol)
MS (ESI, *m/z*): 414.8 [M(^{79}Br)+H]$^+$; 416.8 [M(^{81}Br)+H]$^+$

Dibenzyl (2*R*)-3-bromo-2-hydroxypropyl phosphate, (*R*)-58

Following the general procedure 4.2.1.16., bromoalcohol **(*R*)-58** was obtained from oxirane **(*S*)-57** (1 g, 2.99 mmol) in 99% yield. The spectroscopic data are in agreement with those reported for **(*S*)-58**.



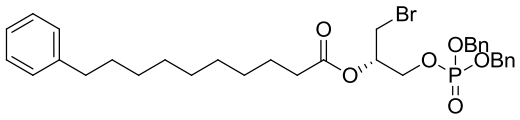
(*R*)-58

$[\alpha]_D^{20}$: +2.2 (c = 0.69, methanol)

4.2.6.2. Synthesis of esters **(*S*)-** and **(*R*)-59**

(1*S*)-2-{[Bis(benzyloxy)phosphoryl]oxy}-1-(bromomethyl)ethyl 10-phenyl decanoate, (*S*)-59

Following the general procedure 4.2.1.17., ester **(*S*)-59** was obtained from carboxylic acid **46** (65 mg, 0.26 mmol) and bromoalcohol **(S)-58** (109 mg, 0.26 mmol) in 68% yield. Chromatography: hexane to hexane/ethyl acetate, 6:4.

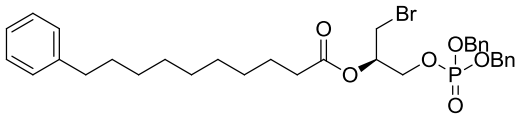


(*S*)-59

R_f: 0.66 (hexane/ethyl acetate, 7:3)
IR (ATR): 1741 (C=O); 1274 (P=O); 1012 (P-O)
^{1}H-NMR (methanol-d_4, δ)(300 MHz): δ 1.22-1.34 (m, 10H, 5CH_2); 1.52-1.63 (m, 4H, 2CH_2); 2.28 (dt, *J* = 7.4, 2.0, 2H, CH_2CO); 2.58 (t, *J* = 7.6, 2H, CH_2Ph); 3.48 (ABX system, *J* = 11.0, 6.0, 5.4, 2H, CH_2Br); 4.17 (m, 2H, CH_2OP); 5.00-5.09 (m, 4H, 2OCH_2Ph); 5.10-5.17 (m, 1H, CH); 7.10-7.16 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar}); 7.33-7.39 (m, 10H, 10CH_{Ar})
^{13}C-NMR (methanol-d_4, δ)(75 MHz): δ 25.9, 30.0, 30.1, 30.25, 30.29, 30.46, 30.49 (7CH_2); 32.7 (CH_2Br); 34.9 (CH_2CO); 36.9 (PhCH_2); 67.9 (d, *J* = 5.5, CH_2OP); 71.0 (d, *J* = 5.9, 2OCH_2Ph); 72.1 (d, *J* = 7.3, CH); 126.6 (CH_{Ar}); 129.2 (6CH_{Ar}); 129.4 (2CH_{Ar}); 129.7 (4CH_{Ar}); 129.8 (2CH_{Ar}); 137.0 (d, *J* = 6.8, 2C_{Ar}); 143.8 (C_{Ar}); 174.1 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 1.45
$[\alpha]_D^{20}$: +0.6 (c = 0.92, methanol)
MS (ESI, *m/z*): 432.0 [M(^{79}Br)-2OCH_2Ph]$^+$; 434.0 [M(^{81}Br)-2OCH_2Ph]$^+$

(1*R*)-2-{[Bis(benzyloxy)phosphoryl]oxy}-1-(bromomethyl)ethyl 10-phenyl decanoate, (*R*)-59

Following the general procedure 4.2.1.17., ester **(*R*)-59** was obtained from carboxylic acid **46** (65 mg, 0.26 mmol) and bromoalcohol **(*R*)-58** (109 mg, 0.26 mmol) in 73% yield. Chromatography: hexane to hexane/ethyl acetate, 6:4. The spectroscopic data are in agreement with those reported for **(*S*)-59**.



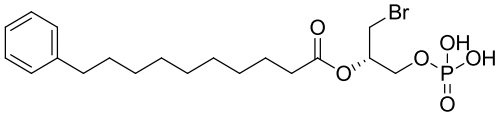
(*R*)-59

$[\alpha]_D^{20}$: -0.1 (c = 1.40, methanol)

4.2.6.3. Synthesis of final compounds **(*S*)-** and **(*R*)-3a**

(1*S*)-2-Bromo-1-[(phosphonooxy)methyl]ethyl 10-phenyldecanoate, (*S*)-3a

Following the general procedure 4.2.1.1., compound **(*S*)-3a** was obtained from dibenzyl phosphate **(*S*)-59** (50 mg, 0.08 mmol) at room temperature in 99% yield. The spectroscopic data are in agreement with those reported for **3a**.

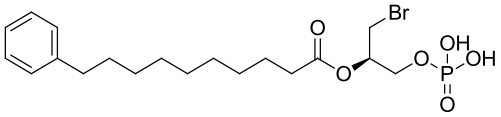


(*S*)-3a

$[\alpha]_D^{20}$: +4.8 (c = 0.50, methanol)

(1*R*)-2-Bromo-1-[(phosphonooxy)methyl]ethyl 10-phenyldecanoate, (*R*)-3a

Following the general procedure 4.2.1.1., compound **(*R*)-3a** was obtained from dibenzyl phosphate **(*R*)-59** (69 mg, 0.11 mmol) at room temperature in 97% yield. The spectroscopic data are in agreement with those reported for its enantiomer **(*S*)-3a**.



(*R*)-3a

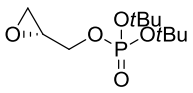
$[\alpha]_D^{20}$: -4.6 (c = 0.50, methanol)

4.2.7. Synthesis of final compounds **3b-d**

4.2.7.1. Synthesis of bromoalcohol **63**

Di-*tert*-butyl (2*R*)-oxiran-2-ylmethyl phosphate, 62

Following the general procedure 4.2.1.13., compound **62** was obtained from (2*S*)-oxiran-2-ylmethanol (0.18 mL, 2.69 mmol) and di-*tert*-butyl *N*,*N*-diisopropylphosphoramidite (1.7 mL, 5.39 mmol) in 52% yield. Chromatography: hexane to ethyl acetate.



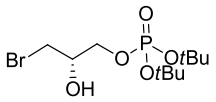
62

R_f: 0.5 (hexane/ethyl acetate, 1:1)
IR (ATR): 1263 (P=O); 999 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.48 (s, 9H, $3CH_3$); 1.49 (s, 9H, $3CH_3$); 2.66 (dd, *J* = 4.7, 2.6, 1H, ½$CH_{2\ epox}$); 2.83 (t, *J* = 4.7, 1H, ½$CH_{2\ epox}$); 3.21-3.27 (m, 1H, CH); 3.85-3.94 (m, 1H, ½CH_2OP); 4.10-4.18 (m, 1H, ½CH_2OP)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 29.9 ($3CH_3$); 30.0 ($3CH_3$); 44.9 ($CH_{2\ epox}$); 50.3 (d, *J* = 9.0, CH); 67.4 (d, *J* = 5.8, CH_2OP); 82.8 (d, *J* = 7.4, C); 82.83 (d, *J* = 7.4, C)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -6.90
$[\alpha]_D^{20}$: -9.2 (c = 1.02, methanol)
MS (ESI, *m/z*): 289.0 $[M+Na]^+$

(2*S*)-3-Bromo-2-hydroxypropyl di-*tert*-butyl phosphate, 63

Following the general procedure 4.2.1.16., bromoalcohol **63** was obtained from oxirane **62** (210 mg, 0.79 mmol) in 95% yield.



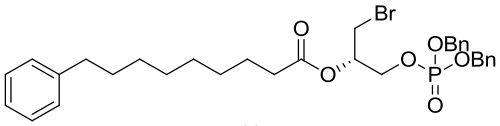
63

R_f: 0.37 (hexane/ethyl acetate, 1:1)
IR (ATR): 3342 (O-H); 1251 (P=O); 996 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.50 (s, 18H, $6CH_3$); 3.49 (d, *J* = 6.0, 2H, CH_2Br); 4.00-4.06 (m, 1H, CH); 4.12-4.17 (m, 2H, CH_2OP); 6.34 (br s, 1H, OH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 29.9 ($3CH_3$); 30.0 ($3CH_3$); 33.4 (CH_2Br); 68.5 (d, *J* = 6.3, CH_2OP); 70.1 (d, *J* = 6.2, CH); 84.5 (d, *J* = 7.4, C); 84.53 (d, *J* = 7.4, C)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -7.05
$[\alpha]_D^{20}$: +3.2 (c = 1.11, methanol)
MS (ESI, *m/z*): 369.0 $[M(^{79}Br)+Na)]^+$; 371.0 $[M(^{81}Br)+Na)]^+$

4.2.7.2. Synthesis of esters **60**, **61**, and **64**

(1*S*)-2-{[Bis(benzyloxy)phosphoryl]oxy}-1-(bromomethyl)ethyl 9-phenyl nonanoate, 60

Following the general procedure 4.2.1.17., ester **60** was obtained from carboxylic acid **45** (122 mg, 0.52 mmol) and bromoalcohol **(*S*)-58** (216 mg, 0.52 mmol) in 96% yield. Chromatography: dichloromethane to dichloromethane/ethyl acetate, 9:1.



60

R_f: 0.90 (dichloromethane/ethyl acetate, 10:1)
IR (ATR): 1741 (C=O); 1269 (P=O); 1015 (P-O)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.27-1.28 (m, 8H, $4CH_2$); 1.52-1.63 (m, 4H, $2CH_2$); 2.28 (dt, *J* = 7.5, 1.8, 2H, CH_2CO); 2.57 (t, *J* = 7.5, 2H, $PhCH_2$); 3.47 (ABX system, *J* = 11.1, 6.0, 5.3, 2H, CH_2Br); 4.17 (m, 2H, CH_2OP); 5.04 (d, *J* = 2.8, 2H,

OC$\underline{H_2}$Ph); 5.07 (d, *J* = 2.9, 2H, OC$\underline{H_2}$Ph); 5.10-5.17 (m, 1H, CH); 7.10-7.15 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar}); 7.31-7.39 (app s, 10H, 10CH_{Ar})

^{13}C-NMR (methanol-*d*$_4$, 75 MHz): δ 24.9, 29.0, 29.1, 29.2, 29.3, 29.4 (6CH_2); 31.7 (CH_2Br); 33.9 ($\underline{C}H_2$CO); 35.9 (Ph$\underline{C}H_2$); 66.9 (d, *J* = 5.5, CH_2OP); 70.0 (d, *J* = 5.9, 2O$\underline{C}H_2$Ph); 71.1 (d, *J* = 7.6, CH); 125.6 (CH_{Ar}); 128.2 (4CH_{Ar}); 128.3 (2CH_{Ar}); 128.4 (2CH_{Ar}); 128.8 (4CH_{Ar}); 128.9 (2CH_{Ar}); 135.95, 136.0, 143.0 (3C_{Ar}); 173.1 (CO)

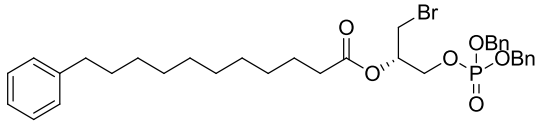
^{31}P-NMR (methanol-*d*$_4$, 121 MHz): δ 1.47

$[\alpha]_D^{20}$: +0.6 (c = 0.92, methanol)

MS (ESI, *m/z*): 631.2 [M(^{79}Br)+H]$^+$; 633.2 [M(^{81}Br)+H]$^+$

(1*S*)-2-{[Bis(benzyloxy)phosphoryl]oxy}-1-(bromomethyl)ethyl 11-phenyl undecanoate, 61

Following the general procedure 4.2.1.17., ester **61** was obtained from 11-phenylundecanoic acid (79 mg, 0.30 mmol) and bromoalcohol **(*S*)-58** (125 mg, 0.30 mmol) in 55% yield. Chromatography: dichloromethane to dichloromethane/ethyl acetate, 9:1.



61

R_f: 0.50 (dichloromethane/ethyl acetate, 10:1)

IR (ATR): 1742 (C=O); 1282 (P=O); 1012 (P-O)

^{1}H-NMR (methanol-*d*$_4$, δ)(300 MHz): δ 1.26-1.29 (m, 12H, 6CH_2); 1.52-1.63 (m, 4H, 2CH_2); 2.29 (dt, *J* = 7.3, 2.0, 2H, CH_2CO); 2.58 (t, *J* = 7.6, 2H, PhC$\underline{H_2}$); 3.48 (ABX system, *J* = 11.0, 5.9, 5.4, 2H, CH_2Br); 4.17 (m, 2H, CH_2OP); 5.04 (d, *J* = 2.8, 2H, OC$\underline{H_2}$Ph); 5.07 (d, *J* = 2.7, 2H, OC$\underline{H_2}$Ph); 5.13 (qt, *J* = 4.9, 1H, CH); 7.10-7.16 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar}); 7.35-7.39 (app s, 10H, 10CH_{Ar})

^{13}C-NMR (methanol-*d*$_4$, δ)(75 MHz): δ 25.9 (CH_2); 30.1 (2CH_2); 30.29, 30.31, 30.5, 30.54, 30.6 (5CH_2); 32.8 (CH_2Br); 34.9 ($\underline{C}H_2$CO); 36.9 (Ph$\underline{C}H_2$); 67.9 (d, *J* = 6.0, CH_2OP); 71.06 (d, *J* = 5.9, O$\underline{C}H_2$Ph); 71.07 (d, *J* = 6.0, O$\underline{C}H_2$Ph); 72.1 (d, *J* = 7.6, CH); 126.6 (CH_{Ar}); 129.2 (6CH_{Ar}); 129.4 (2CH_{Ar}); 129.7 (4CH_{Ar}); 129.9 (2CH_{Ar}); 137.0, 137.1, 144.0 (3C_{Ar}); 174.2 (CO)

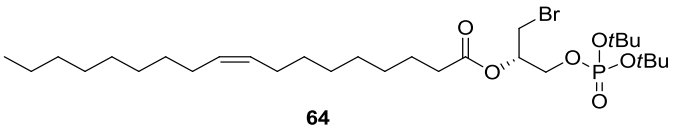
^{31}P-NMR (methanol-*d*$_4$, 121 MHz): δ 1.44

$[\alpha]_D^{20}$: -2.3 (c = 0.59, methanol)

MS (ESI, *m/z*): 659.2 [M(^{79}Br)+H]$^+$; 661.2 [M(^{81}Br)+H]$^+$

(1*S*)-2-Bromo-1-{[(di-*tert*-butoxyphosphoryl)oxy]methyl}ethyl (9*Z*)-octadec-9-enoate, 64

To a stirred solution of bromoalcohol **63** (80 mg, 0.23 mmol, 1.1 equiv) in 1.5 mL of anhydrous dichloromethane at room temperature, pyridine (34 µL, 0.42 mmol, 2 equiv) was added, followed by oleoyl chloride (69 µL, 0.21 mmol, 1 equiv). The reaction mixture was stirred overnight at room temperature. Afterward, the reaction mixture was concentrated under reduced pressure and purified by flash chromatography (alumina, hexane to hexane/ethyl acetate, 10:1) to afford pure ester **64** in 15% yield.



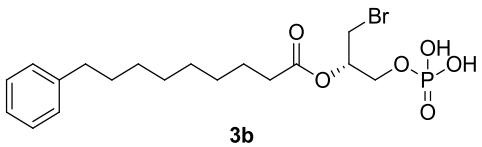
64

R_f: 0.64 (dichloromethane/ethyl acetate, 20:1)
IR (ATR): 1744 (C=O); 1002 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.5, 3H, CH_3); 1.18-1.39 (m, 20H, $10CH_2$); 1.49 (s, 18H, $6CH_3$); 1.57-1.71 (m, 2H, CH_2); 1.95-2.06 (m, 4H, $2CH_2CH_{alkene}$); 2.35 (t, *J* = 7.7, 2H, CH_2CO); 3.52 (dd, *J* = 10.9, 5.2, 1H, ½CH_2Br); 3.61 (dd, *J* = 10.9, 5.4, 1H, ½CH_2Br); 4.05-4.20 (m, 2H, CH_2OP); 5.15 (qt, *J* = 5.2, 1H, CH); 5.27-5.43 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.0 ($2CH_2$); 27.3, 27.4 ($2CH_2CH_{alkene}$); 29.2, 29.24, 29.3 ($3CH_2$); 29.5 ($2CH_2$); 29.7, 29.8, 29.9 ($3CH_2$); 29.95 ($3CH_3$); 30.0 ($3CH_3$); 30.1 (CH_2Br); 32.1 (CH_2); 34.3 (CH_2CO); 65.1 (d, *J* = 5.9, CH_2OP); 70.7 (d, *J* = 9.3, CH); 83.0 (d, *J* = 7.4, C); 83.05 (d, *J* = 7.4, C); 129.9, 130.2 ($2CH_{alkene}$); 172.9 (CO)
^{31}P-NMR ($CDCl_3$, 121 MHz): δ -7.14
$[\alpha]_D^{20}$: +4.9 (c = 0.66, methanol)
MS (ESI, *m/z*): 633.3 [M(^{79}Br)+Na]$^+$; 635.3 [M(^{81}Br)+Na]$^+$

4.2.7.3. Synthesis of final compounds **3b**-**d**

(1*S*)-2-Bromo-1-[(phosphonooxy)methyl]ethyl 9-phenylnonanoate, 3b

Following the general procedure 4.2.1.1., compound **3b** was obtained from dibenzyl phosphate **60** (249 mg, 0.39 mmol) at room temperature in 80% yield.

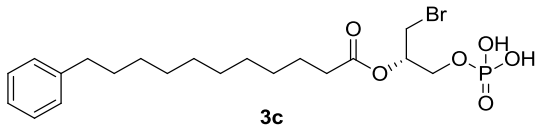


3b

R_f: 0.51 (dichloromethane/ethyl acetate, 10:1)
IR (ATR): 3320 (O-H); 1021 (P-OH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.33 (app s, 8H, 4CH_2); 1.59-1.66 (m, 4H, 2CH_2); 2.38 (t, *J* = 7.4, 2H, CH_2CO); 2.60 (t, *J* = 7.7, 2H, PhCH_2); 3.63 (ABX system, *J* = 11.0, 6.0, 4.9, 2H, CH_2Br); 4.14 (app t, *J* = 5.9, 2H, CH_2OP); 5.17 (qt, *J* = 5.2, 1H, CH); 7.10-7.17 (m, 3H, 3CH_{Ar}); 7.22-7.27 (m, 2H, 2CH_{Ar})
^{13}C-NMR (methanol-d_4, 75 MHz): δ 24.9, 29.1, 29.2, 29.3, 29.4, 29.7 (6CH_2); 31.7 (CH_2Br); 33.9 (CH_2CO); 35.9 (PhCH_2); 65.3 (d, *J* = 5.1, CH_2OP); 71.5 (d, *J* = 8.3, CH); 125.6 (CH_{Ar}); 128.2 (2CH_{Ar}); 128.4 (2CH_{Ar}); 143.0 (C_{Ar}); 173.3 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 2.76
$[\alpha]_D^{20}$: +0.95 (c = 0.57, methanol)
HRMS (ESI, *m/z*): calculated for $C_{18}H_{27}{}^{79}BrO_6P$ ([M(^{79}Br)-H]$^-$): 449.0807, found: 449.0465; calculated for $C_{18}H_{27}{}^{81}BrO_6P$ ([M(^{81}Br)-H]$^-$): 451.0786, found: 451.0611
HPLC (method A, t_R, min): 9.42

(1*S*)-2-Bromo-1-[(phosphonooxy)methyl]ethyl 11-phenylundecanoate, 3c

Following the general procedure 4.2.1.1., compound **3c** was obtained from dibenzyl phosphate **61** (48 mg, 73 µmol) at room temperature in 72% yield.

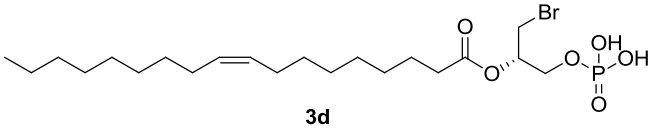


3c

R_f: 0.46 (dichloromethane/ethyl acetate, 10:1)
IR (ATR): 3336 (O-H); 1689 (C=O); 1251 (P=O); 1171 (P-OH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.26-1.36 (m, 12H, 6CH_2); 1.56-1.68 (m, 4H, 2CH_2); 2.37 (t, *J* = 7.3, 2H, CH_2CO); 2.59 (t, *J* = 7.6, 2H, PhCH_2); 3.62 (ABX system, *J* = 10.9, 6.0, 4.8, 2H, CH_2Br); 4.13 (app t, *J* = 5.0, 2H, CH_2OP); 5.16 (qt, *J* = 5.1, 1H, CH); 7.10-7.16 (m, 3H, 3CH_{Ar}); 7.21-7.26 (m, 2H, 2CH_{Ar})
^{13}C-NMR (methanol-d_4, 75 MHz): δ 26.0, 30.1, 30.3, 30.4, 30.5, 30.55, 30.62, 30.8 (8CH_2); 32.8 (CH_2Br); 34.9 (CH_2CO); 36.9 (PhCH_2); 66.3 (d, *J* = 4.9, CH_2OP); 72.5 (d, *J* = 8.8, CH); 126.6 (CH_{Ar}); 129.2 (2CH_{Ar}); 129.4 (2CH_{Ar}); 144.0 (C_{Ar}); 174.3 (CO)
^{31}P-NMR (methanol-d_4, 121 MHz): δ 2.82
$[\alpha]_D^{20}$: -7.8 (c = 0.81, methanol)
HRMS (MALDI, *m/z*): calculated for $C_{20}H_{32}Na^{79}BrO_6P$ ([M(^{79}Br)+Na]$^+$): 501.1018, found: 501.1002; calculated for $C_{20}H_{32}Na^{81}BrO_6P$ ([M(^{81}Br)+Na]$^+$): 503.0997, found: 503.1019
HPLC (method A, t_R, min): 10.06

(1*S*)-2-Bromo-1-[(phosphonooxy)methyl]ethyl (9*Z*)-octadec-9-enoate, 3d

Following the general procedure 4.2.1.8., compound **3d** was obtained from di-*tert*-butyl phosphate **64** (5 mg, 8.17 µmol) and TFA (15 µL, 204 µmol) without further purification in 81% yield.



3d

IR (ATR): 1739 (C=O); 1666 (C=C); 1071 (P-O)

^{1}H-NMR (methanol-d_4, 700 MHz): δ 0.90 (t, *J* = 7.0, 3H, CH_3); 1.28-1.35 (m, 20H, $10CH_2$); 1.62-1.65 (m, 2H, CH_2CH_2CO); 2.02-2.05 (m, 4H, $2CH_2CH_{alkene}$); 2.37 (td, *J* = 7.4, 3.2, 2H, CH_2CO); 3.60 (dd, *J* = 11.0, 6.1, 1H, ½CH_2Br); 3.68 (dd, *J* = 11.0, 4.6, 1H, ½CH_2Br); 4.07-4.12 (m, 2H, CH_2OP); 5.14-5.18 (m, 1H, CH); 5.32-5.37 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR (methanol-d_4, 175 MHz): δ 14.5 (CH_3); 23.8, 26.0 ($2CH_2$); 28.1 ($2CH_2CH_{alkene}$); 30.1, 30.2, 30.3, 30.4, 30.5, 30.6, 30.8, 30.9, 31.1, 33.1 ($10CH_2$); 35.0 (CH_2CO); 66.0 (d, *J* = 3.1, CH_2OP); 72.8 (d, *J* = 5.5, CH); 130.8, 130.9 ($2CH_{alkene}$); 174.4 (CO)

^{31}P-NMR (methanol-d_4, 202 MHz): δ 3.36

$[\alpha]_D^{20}$: +3.3 (c = 0.33, methanol)

HRMS (ESI, *m/z*): calculated for $C_{21}H_{39}{}^{79}BrO_6P$ ([M(^{79}Br)-H]$^-$): 497.1673, found: 497.1664; calculated for $C_{21}H_{39}{}^{81}BrO_6P$ ([M(^{81}Br)-H]$^-$): 499.1653, found: 499.1642

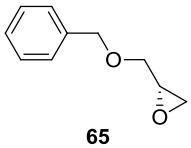
HPLC (method B, t_R, min): 25.58

4.2.8. Synthesis of final compounds **4a**-**e**

4.2.8.1. Synthesis of alcohol **68**

(2*S*)-2-[(Benzyloxy)methyl]oxirane, 65

To a suspension of NaH (65 mg, 1.62 mmol, 1.2 equiv, 60% dispersion in oil) in anhydrous DMF (3 mL) at 0ºC under an argon atmosphere, (2*R*)-oxiran-2-ylmethanol (100 mg, 1.35 mmol, 1 equiv) was added, followed by benzyl bromide (0.21 mL, 1.76 mmol, 1.3 equiv). The reaction was allowed to warm up to room temperature and stirred overnight. Afterward, water was added and the mixture was extracted with dichloromethane. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford product **65** in quantitative yield, which was used in the next step without further purification. Spectroscopic data correspond with those previously reported.[107]

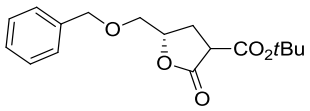


65

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.62 (dd, *J* = 5.0, 2.7, 1H, ½$CH_{2\ epox}$); 2.81 (dd, *J* = 4.9, 4.1, 1H, ½$CH_{2\ epox}$); 3.17-3.22 (m, 1H, CH); 3.45 (dd, *J* = 11.4, 5.8, 1H, ½CH_2O); 3.77 (dd, *J* = 11.4, 3.0, 1H, ½CH_2O); 4.56 (d, *J* = 11.9, 1H, ½OCH_2Ph); 4.62 (d, *J* = 11.9, 1H, ½OCH_2Ph); 7.27-7.39 (m, 5H, $5CH_{Ar}$)

tert-Butyl (5*S*)-5-((benzyloxy)methyl)-2-oxotetrahydrofuran-3-carboxylate, 66

Di-*tert*-butyl malonate (0.97 mL, 1.58 mmol, 2 equiv) was added dropwise to a stirred suspension of NaH (63 mg, 1.58 mmol, 2 equiv, 60% dispersion in oil) in a 2:1 mixture of anhydrous DMF:THF (6 mL) at 0°C, and the mixture was stirred at room temperature for 30 min under an argon atmosphere. A solution of **65** (130 mg, 0.79 mmol, 1 equiv) in dry THF (2 mL) was then added, and the resulting mixture was stirred at 80°C for 6 h. After cooling to room temperature, the reaction was quenched by addition of a saturated aqueous solution of NH_4Cl, diluted with water, and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (dichloromethane) to afford lactone **66** in 62% yield.



66

R_f: 0.36 (hexane/ethyl acetate, 9:1)

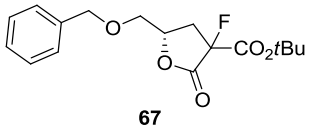
IR (ATR): 1777 (C=O); 1729 (C=O); 1147 (C-O)

^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture of diastereoisomers A:B (3:2): δ 1.48 (s, 9H, $3CH_{3\ B}$); 1.49 (s, 9H, $3CH_{3\ A}$); 2.36 (ABX system, *J* = 13.0, 9.7, 4.7, 2H, $CH_2CHCO_2tBu_{\ B}$); 2.42-2.49 (m, 1H, ½$CH_2CHCO_2tBu_{\ A}$); 2.60-2.70 (m, 1H, ½$CH_2CHCO_2tBu_{\ A}$); 3.47-3.71 (m, 3H, $CHCO_2tBu$, OCH_2CH); 4.50-4.65 (m, 3H, $PhCH_2$, $OCH_2CH_{\ B}$); 4.71-4.78 (m, 1H, $OCH_2CH_{\ A}$); 7.26-7.38 (m, 5H, $5CH_{Ar}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture of diastereoisomers A:B (3:2): δ 28.0 ($3CH_3$); 28.3 ($CH_2CHCO_2tBu_{\ B}$); 28.5 ($CH_2CHCO_2tBu_{\ A}$); 47.6 ($CHCO_2tBu_{\ B}$); 47.8 ($CHCO_2tBu_{\ A}$); 71.2 ($PhCH_2$); 73.6 ($OCH_2CH_{\ B}$); 73.7 ($OCH_2CH_{\ A}$); 77.7 ($OCH_2CH_{\ B}$); 77.8 ($OCH_2CH_{\ A}$); 82.8 (C_A); 82.9 (C_B); 127.7 ($2CH_{Ar\ A}$); 127.8 ($2CH_{Ar\ B}$); 127.9 ($CH_{Ar\ B}$); 128.0 ($CH_{Ar\ A}$); 128.6 ($2CH_{Ar\ B}$); 128.62 ($2CH_{Ar\ A}$); 137.6 ($C_{Ar\ A}$); 137.7 ($C_{Ar\ B}$); 166.8 ($CO_2tBu_{\ B}$); 167.3 ($CO_2tBu_{\ A}$); 171.9 ($CO_{lactone\ B}$); 172.6 ($CO_{lactone\ A}$)

***tert*-Butyl (5*S*)-5-[(benzyloxy)methyl]-3-fluoro-2-oxotetrahydrofuran-3-carboxylate, 67**

Following the general procedure 4.2.1.5., compound **67** was obtained from lactone **66** (1.70 g, 5.54 mmol) in 99% yield, and it was used in the next step without further purification.



67

R_f: 0.80 (dichloromethane)

IR (ATR): 1794 (C=O); 1756 (C=O); 1157 (C-F)

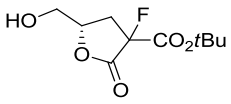
^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture of diastereoisomers A:B (2.3:1): δ 1.48 (s, 9H, $3CH_{3\,B}$); 1.50 (s, 9H, $3CH_{3\,A}$); 2.48-2.81 (m, 2H, CH_2CF); 3.60-3.76 (m, 2H, OCH_2CH); 4.59 (s, 2H, $PhCH_2$); 4.69-4.78 (m, 1H, CH_A); 4.81-4.89 (m, 1H, CH_B); 7.29-7.38 (m, 5H, $5CH_{Ar}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture of diastereoisomers A:B (2.3:1): δ 27.8 ($3CH_{3\,B}$); 27.9 ($3CH_{3\,A}$); 35.1 (d, *J* = 21.9, CH_2CF_B); 35.2 (d, *J* = 22.4, CH_2CF_A); 69.8 ($PhCH_2$); 73.7 (OCH_2CH_B); 73.8 (OCH_2CH_A); 76.5 (d, *J* = 4.9, CH_A); 76.6 (CH_B); 85.3 ($C(CH_3)_{3\,B}$); 85.6 ($C(CH_3)_{3\,A}$); 127.7 ($2CH_{Ar\,B}$); 127.8 ($2CH_{Ar\,A}$); 128.0 ($CH_{Ar\,B}$); 128.1 ($CH_{Ar\,A}$); 128.56 ($2CH_{Ar\,B}$); 128.59 ($2CH_{Ar\,A}$); 137.2 ($C_{Ar\,A}$); 137.5 ($C_{Ar\,B}$); 168.5 (d, *J* = 24.8, $CO_{lactone}$); CF and CO_2tBu not observed

MS (ESI, *m/z*): 342.1 $[M+NH_4]^+$

***tert*-Butyl (5*S*)-3-fluoro-5-(hydroxymethyl)-2-oxotetrahydrofuran-3-carboxylate, 68**

Following the general procedure 4.2.1.1., alcohol **68** was obtained from **67** (480 mg, 1.48 mmol) at 60ºC in quantitative yield.



68

R_f: 0.25 (dichloromethane)

IR (ATR): 3318 (O-H); 1689 (C=O)

^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture of diastereoisomers A:B (1.2:1): δ 1.52 (s, 9H, $3CH_3$); 2.51-2.95 (m, 2H, CH_2CF); 3.66-3.75 (m, 1H, ½CH_2OH); 3.96-4.03 (m, 1H, ½CH_2OH); 4.67-4.75 (m, 1H, CH_B); 4.78-4.86 (m, 1H, CH_A)

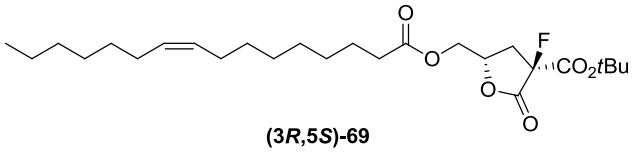
^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture of diastereoisomers A:B (1.2:1): δ 27.9 ($3CH_3$); 34.2 (d, *J* = 22.4) and 34.3 (d, *J* = 21.9, CH_2CF); 62.5 and 62.8 (CH_2OH); 78.2 (d, *J* = 4.7) and 78.9 (CH); 85.5 and 85.7 ($C(CH_3)_3$); 92.2 (d, *J* = 198.0) and 92.4 (d, *J* = 199.0, CF); 164.5 (d, *J* = 26.9, CO_2tBu); 168.7 (d, *J* = 25.0, $CO_{lactone}$)

MS (ESI, *m/z*): 177.9 [M-*t*Bu]$^+$; 159.9 [M-F-*t*Bu]$^+$

4.2.8.2. Synthesis of esters **(3*R*, 5*S*)-** and **(3*S*, 5*S*)-69**, **70**, **72**, **74** and **75**

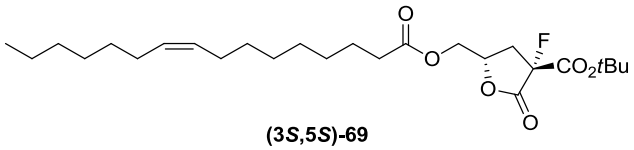
***tert*-Butyl (3*R*,5*S*)-3-fluoro-5-{[(9*Z*)-hexadec-9-enoyloxy]methyl}-2-oxotetrahydrofuran-3-carboxylate, (3*R*,5*S*)-69 and *tert*-butyl (3*S*,5*S*)-3-fluoro-5-{[(9*Z*)-hexadec-9-enoyloxy]methyl}-2-oxotetrahydrofuran-3-carboxylate, (3*S*,5*S*)-69**

Following the general procedure 4.2.1.17., **(3*R*,5*S*)-** and **(3*S*,5*S*)-69** were obtained from palmitoleic acid (120 mg, 0.47 mmol) and alcohol **68** (105 mg, 0.45 mmol) in 25% and 21% yield, respectively. Chromatography: hexane to hexane/ethyl acetate, 4:1.



(3*R*,5*S*)-69

R_f: 0.47 (hexane/ethyl acetate, 4:1)
IR (ATR): 1746 (C=O); 1016 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.9, 3H, CH_3); 1.23-1.37 (m, 16H, $8CH_2$); 1.53 (s, 9H, $3CH_3$); 1.59-1.67 (m, 2H, CH_2CH_2CO); 1.99-2.03 (m, 4H, $2CH_2CH_{alkene}$); 2.37 (t, *J* = 7.6, 2H, CH_2CO); 2.44 (ddd, *J* = 22.9, 13.9, 8.5, 1H, ½CH_2CF); 2.86 (dt, *J* = 13.9, 7.0, 1H, ½CH_2CF); 4.21 (dd, *J* = 12.6, 5.1, 1H, ½$COCH_2$); 4.42 (dd, *J* = 12.6, 2.9, 1H, ½CO_2CH_2); 4.79-4.86 (m, 1H, CH); 5.30-5.39 (m, 2H, $2CH_{alkene}$)
The following NOE effects were observed when the peak at δ 4.79-4.86 (m, CH) was irradiated: 1.53 (s, $3CH_3$); 2.44 (ddd, ½CH_2CF); 4.42 (dd, ½CO_2CH_2).
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 24.9, 27.3, 27.4 ($4CH_2$); 28.0 ($3CH_3$); 29.1, 29.21, 29.22, 29.3, 29.8, 29.9, 31.9, 34.1 ($8CH_2$); 35.2 (d, *J* = 22.1, CH_2CF); 63.6 (CO_2CH_2); 75.1 (d, *J* = 4.6, 1H, CH); 85.9 ($C(CH_3)_3$); 91.4 (d, *J* = 206.7, CF); 129.9, 130.2 ($2CH_{alkene}$); 164.3 (d, *J* = 28.4, CO_2*t*Bu); 168.1 (d, *J* = 25.1, $CO_{lactone}$); 173.4 (CO)
^{19}F-NMR ($CDCl_3$, 300 MHz): δ -160.4 (dd, *J* = 23.2, 6.7)
$[\alpha]_D^{20}$: +4.4 (c = 0.13, methanol)
HRMS (ESI, *m/z*): calculated for $C_{26}H_{42}FO_6$ ([M-H]$^-$): 469.2960, found: 469.2939



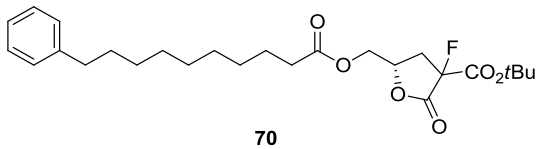
(3*S*,5*S*)-69

R_f: 0.53 (hexane/ethyl acetate, 4:1)
IR (ATR): 1745 (C=O); 1119 (C-F)

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, *J* = 6.7, 3H, CH_3); 1.23-1.36 (m, 16H, $8CH_2$); 1.53 (s, 9H, $3CH_3$); 1.57-1.71 (m, 2H, CH_2CH_2CO); 1.97-2.03 (m, 4H, $2CH_2CH_{alkene}$); 2.33-2.38 (m, 2H, CH_2CO); 2.61 (app dd, *J* = 7.5, 4.0, 1H, ½CH_2CF); 2.70 (app dd, *J* = 7.5, 1.1, 1H, ½CH_2CF); 4.24 (dd, *J* = 12.4, 6.2, 1H, ½$COCH_2$); 4.40 (dd, *J* = 12.4, 3.6, 1H, ½CO_2CH_2); 4.91 (ddd, *J* = 15.1, 7.2, 3.5, 1H, CH); 5.26-5.42 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 22.8, 24.9, 27.3, 27.34 ($4CH_2$); 28.0 ($3CH_3$); 29.1 (CH_2); 29.2 ($2CH_2$); 29.3, 29.8, 29.9, 31.9, 34.1 ($5CH_2$); 35.1 (d, *J* = 22.8, CH_2CF); 63.9 (CO_2CH_2); 75.7 (CH); 85.7 ($C(CH_3)_3$); 91.7 (d, *J* = 199.3, CF); 129.8, 130.2 ($2CH_{alkene}$); 164.4 (d, *J* = 26.7, CO_2tBu); 168.1 (d, *J* = 23.7, $CO_{lactone}$); 179.1 (CO)
^{19}F-NMR ($CDCl_3$, 300 MHz): δ -160.6 (dd, *J* = 27.0, 25.4)
$[\alpha]_D^{20}$: +62.4 (c = 0.03, methanol)
HRMS (ESI, *m/z*): calculated for $C_{26}H_{42}FO_6$ ($[M-H]^-$): 469.3000, found: 469.2939

tert-Butyl (5*S*)-3-fluoro-2-oxo-5-{[(10-phenyldecanoyl)oxy]methyl} tetrahydrofuran-3-carboxylate, 70

Following the general procedure 4.2.1.17., ester **70** was obtained from carboxylic acid **46** (64 mg, 0.26 mmol) and alcohol **68** (60 mg, 0.26 mmol) in 35% yield. Chromatography: dichloromethane.

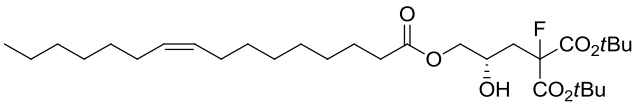


70

R_f: 0.85 (dichloromethane)
IR (ATR): 1743 (C=O); 1156 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): Mixture of diastereoisomers A:B (1.7:1): δ 1.28-1.29 (m, 10H, $5CH_2$); 1.53 (s, 9H, $3CH_{3\,B}$); 1.54 (s, 9H, $3CH_{3\,A}$); 1.58-1.63 (m, 4H, $2CH_2$); 2.36 (t, *J* = 7.6, 2H, CH_2CO); 2.41-2.91 (m, 2H, CH_2CF); 2.59 (t, *J* = 7.8, 2H, $PhCH_2$); 4.19 (dd, *J* = 10.5, 5.1, 1H, ½$CO_2CH_{2\,B}$); 4.24 (dd, *J* = 11.4, 5.4, 1H, ½$CO_2CH_{2\,A}$); 4.38-4.44 (m, 1H, ½CO_2CH_2); 4.79-4.86 (m, 1H, CH_B); 4.87-4.96 (m, 1H, CH_A); 7.16-7.18 (m, 3H, $3CH_{Ar}$); 7.24-7.30 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): Mixture of diastereoisomers A:B (1.7:1): δ 24.9 (CH_2); 28.0 ($3CH_3$); 29.2, 29.3, 29.4, 29.5, 29.6, 31.6 ($6CH_2$); 34.1 (CH_2CO); 35.1 (d, *J* = 22.8) and 35.14 (d, *J* = 21.8, CH_2CF); 36.1 ($PhCH_2$); 63.6 and 63.9 (CO_2CH_2); 75.1 (d, *J* = 4.6) and 75.7 (CH); 85.7 and 85.9 ($C(CH_3)_3$); 125.7 (CH_{Ar}); 128.4 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.02 and 143.04 (C_{Ar}); 164.3 (CO_2tBu); 167.8 (d, *J* = 23.8, $CO_{lactone}$); 173.4 and 173.45 (CO); CF not observed
MS (ESI, *m/z*): 482.3 $[M+NH_4]^+$

Di-*tert*-butyl fluoro{(2*S*)-3-[(9*Z*)-hexadec-9-enoyloxy]-2-hydroxypropyl} propanedioate, 71

Following the general procedure 4.2.1.14., ester **71** was obtained from palmitoleic acid (106 µL, 0.37 mmol) and alcohol **7** (230 mg, 0.75 mmol) in 44% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.

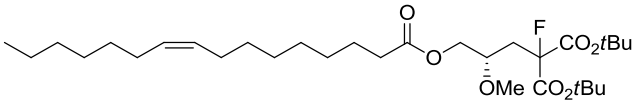


71

R_f: 0.61 (hexane/ethyl acetate, 8:2)
IR (ATR): 3401 (O-H); 1744 (C=O); 1150 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.86 (t, *J* = 6.6, 3H, CH_3); 1.28 (m, 16H, $8CH_2$); 1.48 (s, 18H, $6CH_3$); 1.58-1.63 (m, 2H, CH_2CH_2CO); 1.96-2.02 (m, 4H, $2CH_2CH_{alkene}$); 2.16-2.49 (m, 2H, CH_2CF); 2.32 (t, *J* = 7.5, 2H, CH_2CO); 3.97-4.18 (m, 3H, CH, CO_2CH_2); 5.27-5.38 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 22.8, 25.0 ($2CH_2$); 27.2, 27.3 ($2CH_2CH_{alkene}$); 27.8 ($3CH_3$); 27.9 ($3CH_3$); 29.1, 29.19, 29.2, 29.3, 29.8, 29.83, 31.9 ($7CH_2$); 34.2 (CH_2CO); 37.5 (d, *J* = 20.7, CH_2CF); 65.3 (d, *J* = 3.5, CH); 68.0 (CO_2CH_2); 83.9, 84.0 ($2C(CH_3)_3$); 93.1 (d, *J* = 198.2, CF); 129.8, 130.1 ($2CH_{alkene}$); 165.3 (d, *J* = 24.6, CO_2tBu); 165.7 (d, *J* = 25.8, CO_2tBu); 173.9 (CO)
$[\alpha]_D^{20}$: +6.7 (c = 1.04, methanol)
MS (ESI, *m/z*): 562.4 $[M+NH_4]^+$; 567 $[M+Na]^+$

Di-*tert*-butyl fluoro{(2*S*)-3-[(9*Z*)-hexadec-9-enoyloxy]-2-methoxypropyl} propanedioate, 72

Following the general procedure 4.2.1.9., compound **72** was obtained from **71** (86 mg, 0.16 mmol) in 27% yield. Chromatography: hexane to hexane/ethyl acetate, 8:2.



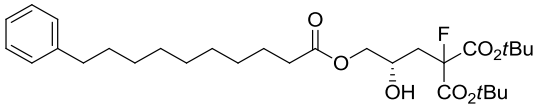
72

R_f: 0.81 (hexane/ethyl acetate, 8:2)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.7, 3H, CH_3); 1.29 (m, 16H, $8CH_2$); 1.48 (s, 9H, $3CH_3$); 1.49 (s, 9H, $3CH_3$); 1.60-1.64 (m, 2H, CH_2CH_2CO); 1.97-2.01 (m, 4H, $2CH_2CH_{alkene}$); 2.17-2.50 (m, 2H, CH_2CF); 2.33 (t, *J* = 7.6, 2H, CH_2CO); 3.30 (s, 3H, OCH_3); 3.57-3.64 (m, 1H, CH); 4.05 (dd, *J* = 11.6, 4.9, 1H, ½CO_2CH_2); 4.17 (dd, *J* = 11.6, 4.7, 1H, ½CO_2CH_2); 5.28-5.39 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 (CH_3); 22.8, 25.0 ($2CH_2$); 27.3, 27.4 ($2\underline{C}H_2CH_{alkene}$); 27.9 ($3CH_3$); 27.91 ($3CH_3$); 29.1 ($CH_2$); 29.2 ($2CH_2$); 29.3, 29.8, 29.9, 31.9 ($4CH_2$); 34.3 ($\underline{C}H_2CO$); 36.7 (d, *J* = 21.2, $\underline{C}H_2CF$); 57.8 (OCH_3); 64.9 ($CO_2\underline{C}H_2$); 74.0 (d, *J* = 3.4, CH); 83.2, 83.6 ($2\underline{C}(CH_3)_3$); 92.6 (d, *J* = 195.3, CF); 129.9, 130.1 ($2CH_{alkene}$); 165.2 (d, *J* = 27.3, $\underline{C}O_2t$Bu); 165.2 (d, *J* = 24.0, $\underline{C}O_2t$Bu); 173.7 (CO)

Di-*tert*-butyl fluoro{(2*S*)-2-hydroxy-3-[(10-phenyldecanoyl)oxy]propyl} propanedioate, 73

Following the general procedure 4.2.1.14., ester **73** was obtained from carboxylic acid **46** (65 mg, 0.26 mmol) and alcohol **7** (161 mg, 0.52 mmol) in 75% yield. Chromatography: dichloromethane to dichloromethane/methanol, 99:1.

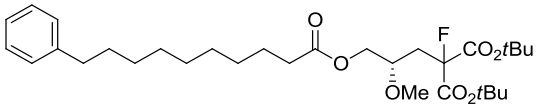


73

$\underline{R_f}$: 0.43 (hexane/ethyl acetate, 8:2)
IR (ATR): 3387 (O-H); 1743 (C=O); 1154 (C-F)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.29 (m, 10H, $5CH_2$); 1.50 (s, 18H, $6CH_3$); 1.56-1.62 (m, 4H, $2CH_2$); 2.17-2.44 (m, 2H, CH_2CF); 2.33 (t, *J* = 7.5, 2H, CH_2CO); 2.59 (t, *J* = 7.8, 2H, $PhC\underline{H}_2$); 4.00-4.20 (m, 3H, CH, CO_2CH_2); 7.17 (d, *J* = 6.5, 3H, $3CH_{Ar}$); 7.24-7.27 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.0 (CH_2); 27.9 ($3CH_3$); 28.0 ($3CH_3$); 29.2, 29.4, 29.41, 29.5, 29.6, 31.6 ($6CH_2$); 34.3 ($\underline{C}H_2CO$); 36.1 ($Ph\underline{C}H_2$); 37.5 (d, *J* = 20.8, $\underline{C}H_2CF$); 65.4 (d, *J* = 3.5, CH); 68.0 ($CO_2\underline{C}H_2$); 83.9, 84.0 ($2\underline{C}(CH_3)_3$); 93.2 (d, *J* = 196.7, CF); 125.7 (CH_{Ar}); 128.3 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.0 (C_{Ar}); 165.2 (d, *J* = 24.6, $\underline{C}O_2t$Bu); 165.6 (d, *J* = 25.9, $\underline{C}O_2t$Bu); 173.9 (CO)

Di-*tert*-butyl fluoro{(2*S*)-2-methoxy-3-[(10-phenyldecanoyl)oxy]propyl} propanedioate, 74

Following the general procedure 4.2.1.9., compound **74** was obtained from **73** (100 mg, 0.19 mmol) in 30% yield. Chromatography: hexane to hexane/ethyl acetate, 8:2.



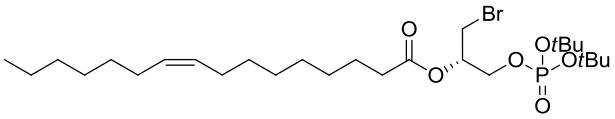
74

$\underline{R_f}$: 0.73 (hexane/ethyl acetate, 8:2)
IR (ATR): 1707 (C=O); 1163 (C-F)

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.25-1.29 (m, 10H, $5CH_2$); 1.48 (s, 9H, $3CH_3$); 1.49 (s, 9H, $3CH_3$); 1.58-1.62 (m, 4H, $2CH_2$); 2.16-2.50 (m, 2H, CH_2CF); 2.32 (t, *J* = 7.5, 2H, CH_2CO); 2.59 (t, *J* = 7.7, 2H, $PhCH_2$); 3.30 (s, 3H, OCH_3); 3.57-3.65 (m, 1H, CH); 4.05 (dd, *J* = 11.7, 4.8, 1H, ½CO_2CH_2); 4.17 (dd, *J* = 11.7, 4.6, 1H, ½CO_2CH_2); 7.14-7.19 (m, 3H, $3CH_{Ar}$); 7.24-7.29 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.0 (CH_2); 27.9 ($3CH_3$); 27.92 ($3CH_3$); 29.3, 29.4, 29.42, 29.5, 29.6, 31.6 ($6CH_2$); 34.3 (CH_2CO); 36.1 ($PhCH_2$); 36.7 (d, *J* = 21.4, CH_2CF); 57.8 (OCH_3); 64.9 (CO_2CH_2); 74.0 (d, *J* = 3.4, CH); 83.4, 83.6 ($2C(CH_3)_3$); 92.6 (d, *J* = 195.4, CF); 125.7 (CH_{Ar}); 128.4 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.0 (C_{Ar}); 165.2 (d, *J* = 27.5 CO_2*t*Bu); 165.24 (d, *J* = 23.9, CO_2*t*Bu); 173.7 (CO)
$[\alpha]_D^{20}$: +3.0 (c = 1.69, methanol)
MS (MALDI, *m/z*): 570.9 $[M+NH_4]^+$

(2*S*)-1-Bromo-3-[(di-*tert*-butoxyphosphoryl)oxy]propan-2-yl (9*Z*)-hexadec-9-enoate, 75

Following the general procedure 4.2.1.17., ester **75** was obtained from palmitoleic acid (0.25 mL, 0.85 mmol) and alcohol **63** (300 mg, 0.85 mmol) in 55% yield. Chromatography: hexane to hexane/ethyl acetate, 6:1.



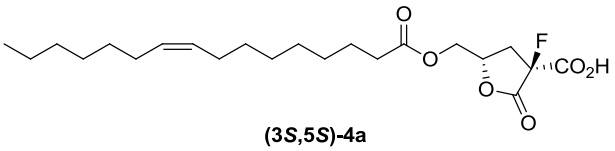
75

R_f: 0.38 (hexane/ethyl acetate, 8:2)
IR (ATR): 1703 (C=O); 1003 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.81 (t, *J* = 6.7, 3H, CH_3); 1.17-1.28 (m, 16H, $8CH_2$); 1.42 (s, 18H, $6CH_3$); 1.52-1.61 (m, 2H, CH_2CH_2CO); 1.90-1.96 (m, 4H, $2CH_2CH_{alkene}$); 2.28 (t, *J* = 7.5, 2H, CH_2CO); 3.45 (dd, *J* = 10.9, 5.2, 1H, ½CH_2Br); 3.54 (dd, *J* = 10.9, 5.5, 1H, ½CH_2Br); 4.02-4.09 (m, 2H, CH_2OP); 5.08 (qt, *J* = 5.2, 1H, CH); 5.22-5.33 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 22.8, 25.0 ($2CH_2$); 27.3, 27.37 ($2CH_2CH_{alkene}$); 29.1, 29.21, 29.23, 29.3, 29.7, 29.8, 29.9 ($7CH_2$); 29.94 ($C(CH_3)_3$); 30.0 ($C(CH_3)_3$); 31.9 (CH_2Br); 34.9 (CH_2CO); 65.1 (d, *J* = 5.9, CH_2OP); 70.7 (d, *J* = 9.2, CH); 83.0 (d, *J* = 7.2, C); 83.1 (d, *J* = 7.6, C); 129.9, 130.2 ($2CH_{alkene}$); 172.9 (CO)
HRMS (ESI, *m/z*): calculated for $C_{27}H_{52}Na^{79}BrO_6P$ ($[M(^{79}Br)+Na]^+$): 605.2585, found: 605.2602; calculated for $C_{27}H_{52}Na^{81}BrO_6P$ ($[M(^{81}Br)+Na]^+$): 607.2581, found: 607.2582

4.2.8.3. Synthesis of final compounds **4a**-**e**

(3*S*,5*S*)-3-Fluoro-5-{[(9*Z*)-hexadec-9-enoyloxy]methyl}-2-oxotetrahydrofuran-3-carboxylic acid, (3*S*,5*S*)-4a

Following the general procedure 4.2.1.8., compound **(3*S*,5*S*)-4a** was obtained from *tert*-butyl ester **(3*R*,5*S*)-69** (5 mg, 10.5 µmol) and TFA (60.9 µL, 0.79 mmol) in 85% yield.

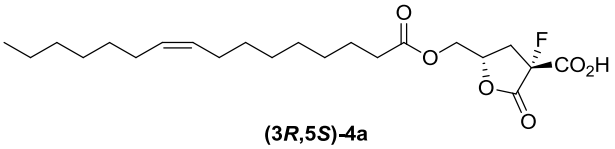


(3*S*,5*S*)-4a

R_f: 0.10 (hexane/ethyl acetate, 1:1)
IR (ATR): 3451 (O-H); 1787 (C=O); 1740 (C=O); 1648 (C=O)
^{1}H-NMR ($CDCl_3$, 700 MHz): δ 0.91 (t, *J* = 6.9, 3H, CH_3); 1.29-1.38 (m, 16H, $8CH_2$); 1.59-1.72 (m, 2H, CH_2CH_2CO); 2.02-2.06 (m, 4H, $2CH_2CH_{alkene}$); 2.39 (t, *J* = 7.5, 2H, CH_2CO); 2.47-2.60 (m, 1H, ½CH_2CF); 2.97-3.09 (m, 1H, ½CH_2CF); 4.26 (dd, *J* = 12.8, 5.1, 1H, ½$COCH_2$); 4.46 (d, *J* = 12.3, 1H, ½CO_2CH_2); 4.92-5.02 (m, 1H, CH); 5.29-5.43 (m, 2H, $2CH_{alkene}$)
^{13}C-NMR ($CDCl_3$, 175 MHz): δ 14.3 (CH_3); 22.8, 24.9, 27.3, 27.4, 29.2, 29.22, 29.3, 29.8, 31.9, 34.1 ($11CH_2$); 34.9 (CH_2CF); 63.5 (CO_2CH_2); 75.5 (CH); 130.1, 130.4 ($2CH_{alkene}$); 167.9 (br s, CO); 173.7 (CO); CF and CO not observed
$[\alpha]_D^{20}$: +12.4 (c = 0.07, methanol)
HRMS (ESI, *m/z*): calculated for $C_{22}H_{34}FO_6$ ($[M-H]^-$): 413.2345, found: 413.2354

(3*R*,5*S*)-3-Fluoro-5-{[(9*Z*)-hexadec-9-enoyloxy]methyl}-2-oxotetrahydrofuran-3-carboxylic acid, (3*R*,5*S*)-4a

Following the general procedure 4.2.1.8., compound **(3*R*,5*S*)-4a** was obtained from *tert*-butyl ester **(3*S*,5*S*)-69** (20 mg, 42 µmol) and TFA (250 µL, 3.19 mmol) in 69% yield.



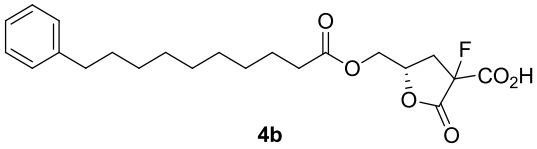
(3*R*,5*S*)-4a

R_f: 0.10 (hexane/ethyl acetate, 1:1)
IR (ATR): 3492 (O-H); 1788 (C=O); 1740 (C=O); 1647 (C=O)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.88 (t, *J* = 6.9, 3H, CH_3); 1.15-1.40 (m, 16H, $8CH_2$); 1.57-1.72 (m, 2H, CH_2CH_2CO); 1.92-2.08 (m, 4H, $2CH_2CH_{alkene}$); 2.35-2.39 (m, 2H, CH_2CO); 2.73 (app d, *J* = 7.5, 1H, ½CH_2CF); 2.79 (app dd, *J* = 7.5, 2.1, 1H, ½CH_2CF); 4.28 (dd, *J* = 12.5, 6.0, 1H, ½$COCH_2$); 4.44 (dd, *J* = 12.5, 3.4, 1H, ½CO_2CH_2); 4.94-4.99 (m 1H, CH); 5.28-5.41 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 14.3 (CH_3); 22.8, 24.9, 27.3, 27.4, 29.1, 29.2, 29.22, 29.3, 29.8, 29.9, 31.9 ($11CH_2$); 34.1 ($\underline{C}H_2CO$); 35.1 (d, *J* = 22.4, $\underline{C}H_2CF$); 63.7 ($CO_2\underline{C}H_2$); 76.1 (CH); 92.0 (d, *J* = 199.8, CF); 129.9, 130.2 ($2CH_{alkene}$); 167.3 (d, *J* = 23.4, CO); 168.6 (d, *J* = 27.2, CO); 173.6 (CO)
$[\alpha]_D^{20}$: +3.0 (c = 0.15, methanol)
HRMS (ESI, *m/z*): calculated for $C_{22}H_{34}FO_6$ ($[M-H]^-$): 413.2345, found: 413.2340

(5*S*)-3-Fluoro-2-oxo-5-{[(10-phenyldecanoyl)oxy]methyl}tetrahydrofuran-3-carboxylic acid, 4b

Following the general procedure 4.2.1.8., compound **4b** was obtained from *tert*-butyl ester **70** (30 mg, 65 µmol) and TFA (0.37 mL, 4.84 mmol) in 90% yield.

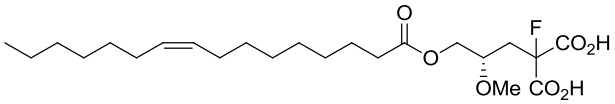


4b

IR (ATR): 3506 (O-H); 1795 (C=O); 1795 (C=O); 1741 (C=O)
^{1}H-NMR ($CDCl_3$, 500 MHz): Mixture of diastereoisomers (1:1): δ 1.26-1.30 (m, 10H, $5CH_2$); 1.59-1.62 (m, 4H, $2CH_2$); 2.36 (t, *J* = 7.3, 2H, CH_2CO); 2.49-3.06 (m, 2H, CH_2CF); 2.59 (t, *J* = 7.7, 2H, $PhC\underline{H}_2$); 4.17-4.30 (m, 1H, ½CO_2CH_2); 4.42-4.49 (m, 1H, ½CO_2CH_2); 4.95-5.03 (m, 1H, CH); 7.16-7.18 (m, 3H, $3CH_{Ar}$); 7.25-7.28 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 125 MHz): Mixture of diastereoisomers (1:1): δ 24.9, 29.2, 29.3, 29.4, 29.5, 29.6, 31.6 ($7CH_2$); 34.0 and 34.1 ($\underline{C}H_2CO$); 35.1 (br d, *J* = 25.0, $\underline{C}H_2CF$); 36.1 ($Ph\underline{C}H_2$); 63.5 and 63.9 ($CO_2\underline{C}H_2$); 75.6 (br s) and 76.3 (CH); 125.6 and 125.7 (CH_{Ar}); 128.4 ($2CH_{Ar}$); 128.5 ($2CH_{Ar}$); 143.0 and 143.1 (C_{Ar}); 168.0 (br s, CO_2H, $CO_{lactone}$); 173.6 and 173.8 (CO); CF not observed
HRMS (ESI, *m/z*): calculated for $C_{22}H_{28}FO_6$ ($[M-H]^-$): 407.1875, found: 407.1875
HPLC (method A, t_R, min): 10.31

Fluoro{(2*S*)-3-[(9*Z*)-hexadec-9-enoyloxy]-2-methoxypropyl}propanedioic acid, 4c

Following the general procedure 4.2.1.8., compound **4c** was obtained from *tert*-butyl ester **72** (20 mg, 36 µmol) and TFA (0.21 mL, 2.68 mmol) in 93% yield.



4c

^{1}H-NMR (methanol-d_4, 300 MHz): δ 0.90 (t, *J* = 6.3, 3H, CH_3); 1.29-1.32 (m, 16H, $8CH_2$); 1.57-1.64 (m, 2H, $C\underline{H}_2CH_2CO$); 2.02-2.04 (m, 4H, $2C\underline{H}_2CH_{alkene}$); 2.10-2.59 (m,

2H, CH_2CF); 2.35 (t, *J* = 7.4, 2H, CH_2CO); 3.32 (s, 3H, OCH_3); 3.60-3.67 (m, 1H, CH); 4.04 (dd, *J* = 11.8, 4.8, 1H, ½CO_2CH_2); 4.25 (dd, *J* = 11.7, 4.1, 1H, ½CO_2CH_2); 5.29-5.39 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR (methanol-d_4, 75 MHz): δ 14.4 (CH_3); 23.7, 26.0 ($2CH_2$); 28.1, 28.2 ($2CH_2CH_{alkene}$); 30.0 (CH_2); 30.1 ($2CH_2$); 30.2, 30.8, 30.82, 32.9 ($4CH_2$); 34.9 (CH_2CO); 38.3 (d, *J* = 21.3, CH_2CF); 58.1 (OCH_3); 65.9 (CO_2CH_2); 75.9 (br s CH); 130.8, 130.9 ($2CH_{alkene}$); 175.2 (CO); $2CO_2H$ and CF not observed

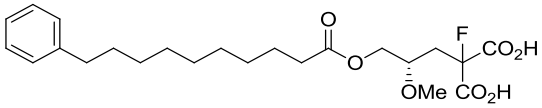
$[\alpha]_D^{20}$: +14.1 (c = 0.79, methanol)

HRMS (ESI, *m/z*): calculated for $C_{23}H_{38}FO_7$ ($[M-H]^-$): 445.2607, found: 445.2618

HPLC (method A, t_R, min): 11.89

Fluoro{(2*S*)-2-methoxy-3-[(10-phenyldecanoyl)oxy]propyl}propanedioic acid, 4d

Following the general procedure 4.2.1.8., compound **4d** was obtained from *tert*-butyl ester **74** (44 mg, 80 µmol) and TFA (0.46 mL, 5.97 mmol) in 68% yield.



4d

IR (ATR): 3342 (O-H); 1736 (C=O))

^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.28-1.31 (m, 10H, $5CH_2$); 1.58-1.62 (m, 4H, $2CH_2$); 2.22-2.54 (m, 2H, CH_2CF); 2.34 (t, *J* = 7.4, 2H, CH_2CO); 2.59 (t, *J* = 7.7, 2H, $PhCH_2$); 3.32 (s, 3H, OCH_3); 3.60-3.65 (m, 1H, CH); 4.04 (dd, *J* = 11.8, 4.8, 1H, ½CO_2CH_2); 4.25 (dd, *J* = 11.7, 4.1, 1H, ½CO_2CH_2); 7.09-7.16 (m, 3H, $3CH_{Ar}$); 7.21-7.26 (m, 2H, $2CH_{Ar}$)

^{13}C-NMR (methanol-d_4, 75 MHz): δ 26.0, 30.1, 30.2, 30.3 ($4CH_2$); 30.5 ($2CH_2$); 32.7 (CH_2); 34.9 (CH_2CO); 36.9 ($PhCH_2$); 38.1 (d, *J* = 21.3, CH_2CF); 58.1 (OCH_3); 65.8 (CO_2CH_2); 75.8 (d, *J* = 3.4, CH); 126.6 (CH_{Ar}); 129.2 ($2CH_{Ar}$); 129.4 ($2CH_{Ar}$); 144.0 (C_{Ar}); 175.2 (CO); $2CO_2H$ and CF not observed

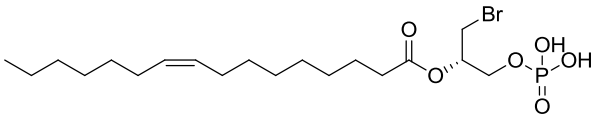
$[\alpha]_D^{20}$: limited solubility

HRMS (ESI, *m/z*): calculated for $C_{23}H_{32}FO_6$ ($[M-H]^-$): 439.2138, found: 439.2119

HPLC (method A, t_R, min): 10.25

(2*S*,9*Z*)-1-Bromo-3-(phosphonooxy)propan-2-yl hexadec-9-enoate, 4e

Following the general procedure 4.2.1.8., compound **4e** was obtained from *tert*-butyl ester **75** (4 mg, 6.9 µmol) and TFA (40 µL, 0.51 mmol) in 93% yield.



4e

IR (ATR): 1741 (C=O); 1457 (C=C); 1015 (P-O)

^{1}H-NMR (methanol-d_4, 500 MHz): δ 0.93 (t, *J* = 6.8, 3H, CH_3); 1.29-1.42 (m, 16H, $8CH_2$); 1.56-1.70 (m, 2H, CH_2CH_2CO); 2.03-2.06 (m, 4H, $2CH_2CH_{alkene}$); 2.40 (t, *J* = 7.4, 2H, CH_2CO); 3.62 (dd, *J* = 10.9, 6.1, 1H, ½CH_2Br); 3.70 (dd, *J* = 10.7, 4.5, 1H, ½CH_2Br); 4.05-4.17 (m, 2H, CH_2OP); 5.13-5.24 (m, 1H, CH); 5.31-5.44 (m, 2H, $2CH_{alkene}$)

^{13}C-NMR (methanol-d_4, 125 MHz): δ 13.4 (CH_3); 22.7 (CH_2); 25.0, 25.1 ($2CH_2CH_{alkene}$); 27.1, 27.13, 27.14, 29.0, 29.2, 29.77, 29.78, 29.8, 31.9 ($9CH_2$); 33.9 (CH_2CO); 60.5 (d, *J* = 7.2, CH_2OP); 64.9 (d, *J* = 5.3, CH); 129.8, 129.9 ($2CH_{alkene}$); 173.4 (CO)

^{31}P-NMR (methanol-d_4, 202 MHz): δ 2.88

$[\alpha]_D^{20}$: +5.9 (c = 0.09, methanol)

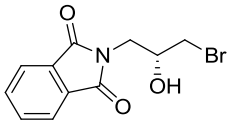
HRMS (ESI, *m/z*): calculated for $C_{19}H_{35}{}^{79}BrO_6P$ ([M(^{79}Br)-H]$^-$): 469.1360, found: 469.1361; calculated for $C_{19}H_{35}{}^{81}BrO_6P$ ([M(^{81}Br)-H]$^-$): 471.1340, found: 471.1342

4.2.9. Synthesis of final compounds **5a**-**b**

4.2.9.1. Synthesis of final compound **5a**

2-[(2*R*)-3-Bromo-2-hydroxypropyl]-1*H*-isoindole-1,3(2*H*)-dione, 76

To a solution of (*R*)-(-)-*N*-(2,3-epoxypropyl)phthalimide (1 g, 4.92 mmol, 1 equiv), in chloroform (6 mL) cooled at 0°C, 48% HBr (7.4 mL) was added dropwise and the reaction was stirred for 30 min. Afterward the mixture was diluted with brine and extracted twice with dichloromethane. The organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure, obtaining **76** without further purification in 96% yield.



76

R_f: 0.73 (hexane/ethyl acetate, 6:4)

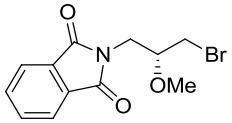
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.82 (br s, 1H, OH); 3.52 (ABX system, *J* = 10.7, 5.5, 4.6, 2H, CH_2Br); 3.92 (ABX system, *J* = 14.2, 7.2, 4.3, 2H, NCH_2); 4.09-4.20 (m, 1H, CH); 7.72-7.78 (m, 2H, $2CH_{Ar}$); 7.85-7.91 (m, 2H, $2CH_{Ar}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 36.6 (CH_2Br); 42.5 (NCH_2); 69.5 (CH); 123.7 ($2CH_{Ar}$); 132.0 ($2C_{Ar}$); 134.4 ($2CH_{Ar}$); 168.8 (2CO)

$[\alpha]_D^{20}$: +2.8 (c = 0.28, methanol)

2-[(2*R*)-3-Bromo-2-methoxypropyl]-1*H*-isoindole-1,3(2*H*)-dione, 77

Following the general procedure 4.2.1.9., compound **77** was obtained from **76** (1.32 g, 4.65 mmol) in 62% yield. Chromatography: hexane to hexane/ethyl acetate, 8:2.

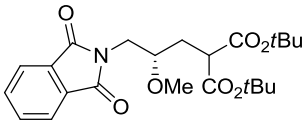


77

R_f: 0.67 (hexane/ethyl acetate, 6:4)
IR (ATR): 1713 (C=O); 1016 (C-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 3.35-3.51 (m, 2H, CH_2Br); 3.42 (m, 3H, CH_3); 3.65-3.93 (m, 3H, NCH_2, CH); 7.68-7.74 (m, 2H, $2CH_{Ar}$); 7.80-7.86 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 32.3 (CH_2Br); 39.9 (NCH_2); 58.0 (CH_3); 78.0 (CH); 123.5 ($2CH_{Ar}$); 131.9 ($2C_{Ar}$); 134.2 ($2CH_{Ar}$); 168.3 (2CO)
$[\alpha]_D^{20}$: +0.96 (c = 0.42, methanol)
MS (ESI, *m/z*): 298.0 [M(^{79}Br)+H]$^+$; 300.0 [M(^{81}Br)+H]$^+$

Di-*tert*-butyl [(2S)-3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-2-methoxypropyl] propanedioate, 78

Di-*tert*-butyl malonate (0.66 mL, 2.94 mmol, 2 equiv) was added dropwise to a stirred suspension of NaH (178 mg, 4.44 mmol, 1.6 equiv, 60% dispersion in oil) in anhydrous DMF (10 mL) under an argon atmosphere. Then, **77** (439 mg, 1.47 mmol, 1 equiv) and potassium iodide (269 mg, 1.62 mmol, 1.1 equiv) were added and the mixture was stirred overnight. Then, the solvent was removed under reduced pressure and the residue was dissolved in DCM and washed with 5% aqueous acetic acid, water and brine. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane to hexane/ethyl acetate, 8:2) to afford **78** in 54% yield.



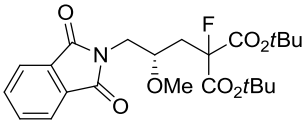
78

R_f: 0.44 (hexane/ethyl acetate, 8:2)
IR (ATR): 1720 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.44 (s, 18H, $6CH_3$); 1.91-2.11 (m, 2H, CH_2CH); 3.37-3.42 (m, 1H, CH_2CH); 3.38 (m, 3H, OCH_3); 3.51-3.59 (m, 1H, $CHOCH_3$); 3.71-3.85 (m, 2H, NCH_2); 7.68-7.74 (m, 2H, $2CH_{Ar}$); 7.82-7.88 (m, 2H, $2CH_{Ar}$)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 28.0 ($6CH_3$); 31.9 ($\underline{C}H_2CH$); 40.4 (NCH_2); 50.6 ($CH_2\underline{C}H$); 57.9 (OCH_3); 76.9 ($\underline{C}HOCH_3$); 81.58, 81.62 ($2\underline{C}(CH_3)_3$); 123.5 ($2CH_{Ar}$); 132.2 ($2C_{Ar}$); 134.1 ($2CH_{Ar}$); 168.4 (2CON); 168.77, 168.80 ($2\underline{C}O$*t*Bu)
$[\alpha]_D^{20}$: -1.1 (c = 0.26, methanol)
MS (ESI, *m/z*): 456.1 $[M+Na]^+$

Di-*tert*-butyl [(2*S*)-3-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-2-methoxypropyl](fluoro)propanedioate, 79

Following the general procedure 4.2.1.5., compound **79** was obtained from **78** (345 mg, 0.80 mmol) in 96% yield, which was used in the next step without further purification.

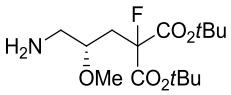


79

R_f: 0.19 (hexane/ethyl acetate, 9:1)
IR (ATR): 1744 (C=O); 1716 (C=O)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.45 (s, 9H, $3CH_3$); 1.47 (s, 9H, $3CH_3$); 2.25-2.45 (m, 2H, CH_2CF); 3.33 (s, 3H, OCH_3); 3.69-3.73 (m, 1H, CH); 3.79-3.80 (m, 2H, NCH_2); 7.71-7.74 (m, 2H, $2CH_{Ar}$); 7.84-7.88 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 27.8 ($3CH_3$); 27.9 ($3CH_3$); 37.8 (*J* = 21.2, $\underline{C}H_2CF$); 40.5 (NCH_2); 57.7 (OCH_3); 74.3 (*J* = 3.1, CH); 83.1, 86.6 ($2\underline{C}(CH_3)_3$); 92.6 (*J* = 195.6, CF); 123.5 ($2CH_{Ar}$); 132.2 ($2C_{Ar}$); 134.2 ($2CH_{Ar}$); 165.16 (*J* = 25.4, $\underline{C}O_2$*t*Bu); 165.17 (*J* = 25.7, $\underline{C}O_2$*t*Bu); 168.4 (2CON)
$[\alpha]_D^{20}$: -1.0 (c = 0.04, methanol)
MS (ESI, *m/z*): 469.2 $[M+NH_4]^+$; 474.2 $[M+Na]^+$

Di-*tert*-butyl [(2*S*)-3-amino-2-methoxypropyl](fluoro)propanedioate, 80

To a solution of phthalimide **79** (378 mg, 0.84 mmol, 1 equiv) in absolute ethanol (8.4 mL), hydrazine monohydrate (1.0 mL, 2.1 mmol 2.5 equiv) was added and the reaction was refluxed for 3 h. Once at room temperature, the mixture was concentrated under reduced pressure, and the residue was dissolved in dichloromethane and washed with 0.5 N NaOH solution. The aqueous phase was extracted with dichloromethane and the combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (triethylamine-neutralized silica gel, dichloromethane to dichloromethane/ethanol, 9:1) affording **80** in 66% yield.

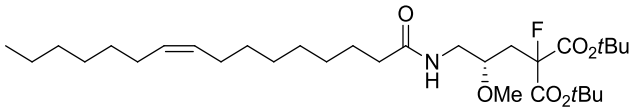


80

R_f: 0.38 (dichloromethane/ethanol, 9:1)
IR (ATR): 3353 (N-H); 1746 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.46 (s, 18H, $6CH_3$); 2.08-2.18 (m, 1H, ½CH_2CF); 2.38 (ddd, *J* = 33.5, 15.2, 8.7, 1H, ½CH_2CF); 2.63-2.68 (m, 1H, ½NH_2CH_2); 2.87-2.93 (m, 1H, ½NH_2CH_2); 3.26 (m, 3H, OCH_3); 3.33-3.40 (m, 1H, CH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.8 ($3CH_3$); 27.9 ($3CH_3$); 36.5 (*J* = 21.0, CH_2CF); 44.4 (NH_2CH_2); 57.2 (OCH_3); 77.0 (CH); 83.2, 83.6 ($2C(CH_3)_3$); 93.0 (*J* = 194.9, CF); 165.3 (*J* = 26.9, CO_2tBu); 165.4 (*J* = 24.4, CO_2tBu)
MS (ESI, *m/z*): 322.2 $[M+H]^+$

Di-*tert*-butyl fluoro{(2*S*)-3-[(9*Z*)-hexadec-9-enoylamino]-2-methoxypropyl} propanedioate, 81

To a solution of palmitoleic acid (22 µL, 78 µmol, 1 equiv) and **80** (35 mg, 0.11 mmol, 1.4 equiv) in anhydrous DMF (1.95 mL) under an argon atmosphere, HOBt (10.5 mg, 78 µmol, 1 equiv) and EDC (17.9 mg, 93 µmol, 1.2 equiv) were added. After stirring for 2 h at room temperature, additional EDC (6 mg, 0.031 mmol, 0.4 equiv) was added and the reaction was stirred overnight. Then, the mixture was transferred to a separating funnel and a 1:1 mixture of dichloromethane/hexane was added. The solution was washed three times with water, followed by 0.5 N NaOH solution, 0.5 N HCl solution, and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane to hexane/ethyl acetate, 7:3) affording **81** in 37% yield.



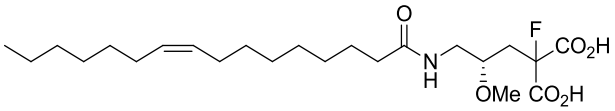
81

R_f: 0.35 (hexane/ethyl acetate, 8:2)
IR (ATR): 3300 (N-H); 1747 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, *J* = 6.6, 3H, CH_3); 1.25-1.34 (m, 16H, $8CH_2$); 1.45-1.49 (m, 18H, $6CH_3$); 1.58-1.69 (m, 2H, CH_2CH_2CO); 1.97-2.05 (m, 4H, $2CH_2CH_{alkene}$); 2.18 (t, *J* = 7.5, 2H, CH_2CO); 2.10-2.21 (m, 1H, ½CH_2CF); 2.34 (ddd, *J* = 13.7, 6.7, 3.5, 1H, ½CH_2CF); 3.28 (s, 3H, OCH_3); 3.39-3.43 (m, 2H, $NHCH_2$); 3.51-3.58 (m, 1H, CH); 5.28-5.39 (m, 2H, $2CH_{alkene}$); 5.63-5.75 (m, 1H, NH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.1 (CH_3); 22.7, 25.8 ($2CH_2$); 27.17, 27.22 ($2CH_2CH_{alkene}$); 27.7 ($3CH_3$); 27.8 ($3CH_3$); 29.0, 29.1, 29.28, 29.3 ($4CH_2$); 29.7 ($2CH_2$); 31.8 (CH_2); 36.77 (d, *J* = 22.1, CH_2CF); 36.8 (CH_2CO); 41.1 (NH_2CH_2); 57.1 (OCH_3);

74.5 (d, *J* = 2.7, CH); 83.1, 83.6 (2C(CH$_3$)$_3$); 92.7 (*J* = 196.4, CF); 129.8, 130.0 (2CH$_{alkene}$); 165.1 (d, *J* = 23.3, CO$_2$*t*Bu); 168.8 (d, *J* = 26.3, CO$_2$*t*Bu); 173.3 (CONH)
HRMS (ESI, *m/z*): calculated for $C_{31}H_{56}FNaNO_6$ ([M+Na]$^+$): 580.3984, found: 580.3992

Fluoro{(2*S*)-3-[(9*Z*)-hexadec-9-enoylamino]-2-methoxypropyl}propanedioic acid, 5a

Following the general procedure 4.2.1.8., compound **5a** was obtained from *tert*-butyl ester **81** (10 mg, 18 µmol) and TFA (104 µL, 1.34 mmol) in 99% yield.



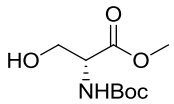
5a

R_f: 0.17 (hexane/ethyl acetate, 6:4)
IR (ATR): 3273 (N-H); 1646 (C=O); 1014 (C-O)
^{1}H-NMR (CDCl$_3$, 700 MHz): δ 0.88 (t, *J* = 7.0, 3H, CH$_3$); 1.25-1.34 (m, 16H, 8CH$_2$); 1.54-1.67 (m, 2H, CH$_2$CH$_2$CO); 1.95-2.04 (m, 4H, 2CH$_2$CH$_{alkene}$); 2.19-2.56 (m, 4H, CH$_2$CO, CH$_2$CF); 3.24-3.70 (m, 6H, NHCH$_2$, CH, OCH$_3$); 5.30-5.36 (m, 2H, 2CH$_{alkene}$)
^{13}C-NMR (CDCl$_3$, 175 MHz): δ 14.3 (CH$_3$); 22.8, 26.0 (2CH$_2$); 27.4 (2CH$_2$CH$_{alkene}$); 29.1, 29.4, 29.5 (3CH$_2$); 29.88 (2CH$_2$); 29.93, 31.9, 36.6 (3CH$_2$); 36.7 (br s, CH$_2$CF); 42.2 (br s, NHCH$_2$); 57.6 (OCH$_3$); 74.5 (CH); 129.8, 130.1 (2CH$_{alkene}$); 169.3 (br s, 2CO$_2$H); 175.6 (CONH); CF not observed
$[\alpha]_D^{20}$: -0.5 (c = 0.14, methanol)
HRMS (ESI, *m/z*): calculated for $C_{23}H_{39}FNO_6$ ([M-H]$^-$): 444.2767, found: 444.2784
HPLC (method A, t_R, min): 29.71

4.2.9.2. Synthesis of aziridine **87**

Methyl *N*-(*tert*-butoxycarbonyl)-D-serinate, 82

To a solution of D-serine methyl ester hydrochloride (500 mg, 3.21 mmol, 1 equiv) and triethylamine (0.97 mL, 6.91 mmol, 2.15 equiv) in anhydrous THF (8 mL) under an argon atmosphere, di-*tert*-butyl dicarbonate (700 mg, 3.21 mmol, 1 equiv) was added at 0ºC. The reaction mixture was heated at 140ºC under MW irradiation for 20 min. Once cooled to room temperature, the solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and washed with 1M HCl solution. The organic phase was washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure, to afford **82** in 99% yield, which was used in next step without further purification. Spectroscopic data correspond with those previously reported.[108]

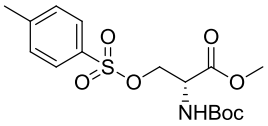


82

R_f: 0.23 (hexane/ethyl acetate, 7:3)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.44 (s, 9H, $3CH_3$); 3.77 (s, 3H, CH_3); 3.91 (qd, *J* = 11.2, 3.7, 2H, CH_2); 4.31-4.44 (m, 1H, CH); 5.53 (d, *J* = 7.3, 1H, NH)
$[\alpha]_D^{20}$: -0.2 (c = 0.30, methanol)

Methyl *N*-(*tert*-butoxycarbonyl)-*O*-[(4-methylphenyl)sulfonyl]-D-serinate, 83

Following the general procedure 4.2.1.3., tosylate **83** was obtained from **82** (720 mg, 3.28 mmol) in 49% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3. Spectroscopic data correspond with those previously reported.[109]

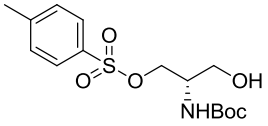


83

R_f: 0.27 (hexane/ethyl acetate, 8:2)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.41 (s, 9H, $3CH_3$); 2.45 (s, 3H, CH_3C_{Ar}); 3.69 (s, 3H, OCH_3); 4.28 (dd, *J* = 10.0, 2.9, 1H, ½CH_2); 4.39 (dd, *J* = 10.1, 2.8, 1H, ½CH_2); 4.48-4.52 (m, 1H, CH); 5.29 (d, *J* = 7.6, 1H, NH); 7.35 (d, *J* = 8.4, 2H, $2CH_{Ar}$); 7.76 (d, *J* = 8.3, 2H, $2CH_{Ar}$)
$[\alpha]_D^{20}$: -0.3 (c = 0.62, methanol)

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-hydroxypropyl 4-methylbenzene sulfonate, 84

To a solution of **83** (500 mg, 1.34 mmol, 1 equiv) in absolute ethanol (4 mL) at -5ºC, a suspension of $NaBH_4$ (142 mg, 3.75 mmol, 2.8 equiv) in an ice/water mixture (1 mL) was added. After stirring 1 h at this temperature, the mixture was brought to pH 4 by dropwise addition of glacial acetic acid and ethanol was removed under reduced pressure. Then the residue was dissolved in ethyl acetate, washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure, affording **84** in 96% yield.

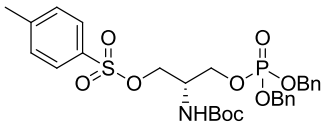


84

R_f: 0.71 (hexane/ethyl acetate, 6:4)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.41 (s, 9H, $3CH_3$); 2.46 (s, 3H, CH_3C_{Ar}); 3.64 (dd, *J* = 11.2, 5.1, 1H, ½OCH_2); 3.76-3.90 (m, 2H, ½OCH_2, CH); 4.06-4.18 (m, 2H, CH_2OH); 4.88-4.98 (m, 1H, NH); 7.36 (d, *J* = 8.0, 2H, $2CH_{Ar}$); 7.79 (d, *J* = 8.3, 2H, $2CH_{Ar}$)
MS (ESI, *m/z*): 245.9 $[M\text{-Boc}+2H]^+$
HPLC (method A, t_R, min): 22.68

(2*S*)-3-{[Bis(benzyloxy)phosphoryl]oxy}-2-[(*tert*-butoxycarbonyl)amino]propyl 4-methylbenzenesulfonate, 85

Following the general procedure 4.2.1.13., compound **85** was obtained **84** (150 mg, 0.43 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.29 mL, 0.87 mmol) in 38% yield. Chromatography: hexane to hexane/ethyl acetate, 6:4.

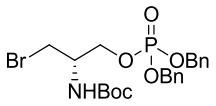


85

R_f: 0.12 (hexane/ethyl acetate, 7:3)
IR (ATR): 1755 (C=O); 1238 (P=O); 1009 (P-O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.40 (s, 9H, $3CH_3$); 2.41 (s, 3H, CH_3C_{Ar}); 3.81-4.10 (m, 5H, CH_2CHCH_2); 4.86-4.96 (m, 1H, NH); 5.00 (s, 2H, CH_2Ph); 5.03 (s, 2H, CH_2Ph); 7.29-7.39 (m, 12H, $12CH_{Ar}$); 7.73-7.76 (m, 2H, $2CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.8 (CH_3C_{Ar}); 28.4 ($3CH_3$); 49.1 (CH); 65.5 (d, *J* = 5.9, CH_2OP); 67.4 (OCH_2); 69.81 (d, *J* = 5.5, CH_2Ph); 69.84 (d, *J* = 5.6, CH_2Ph); 77.4 ($C(CH_3)_3$); 128.1 ($2CH_{Ar}$); 128.15, 128.2, 128.77, 128.8, 128.9 ($10CH_{Ar}$); 130.1 ($2CH_{Ar}$); 132.5 (C_{Ar}); 135.88 (d, *J* = 6.3, C_{Ar}); 135.91 (d, *J* = 6.4, C_{Ar}); 145.3 (C_{Ar}); 155.2 (CO)
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 2.02
$[\alpha]_D^{20}$: -0.9 (c = 0.42, methanol)
MS (ESI, *m/z*): 513.9 $[M\text{-}CH_2Ph]^+$
HPLC (method A, t_R, min): 24.20

***tert*-Butyl (1*S*)-2-{[bis(benzyloxy)phosphoryl]oxy}-1-(bromomethyl)ethyl carbamate, 86**

To a solution of **85** (300 mg, 0.50 mmol, 1 equiv) in acetone (3.5 mL), lithium bromide (86 mg, 0.99 mmol, 2 equiv) was added and the mixture was refluxed for 12 h. Then the solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **86** in 79% yield.



86

R_f: 0.59 (dichloromethane/methanol, 9:1)
IR (ATR): 3402 (NH); 1696 (C=O); 1230 (P=O); 1015 (P-O)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.43 (s, 9H, $3CH_3$); 3.34 (dd, *J* = 10.2, 6.6, 1H, ½CH_2Br); 3.44 (dd, *J* = 10.3, 2.8, 1H, ½CH_2Br); 3.98-4.00 (m, 2H, CH, ½CH_2O); 4.17-4.21 (m, 1H, ½CH_2O); 4.98-4.99 (m, 1H, NH); 5.02-5.10 (m, 4H, $2CH_2$Ph); 7.32-7.38 (app s, 10H, $10CH_{Ar}$)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 28.4 ($3CH_3$); 32.0 (CH_2Br); 50.6 (CH); 66.6 (CH_2O); 69.9 ($2CH_2$Ph); 80.4 (C); 128.2 ($4CH_{Ar}$); 128.8 ($4CH_{Ar}$); 128.9 ($2CH_{Ar}$); 135.7 (d, *J* = 6.4, C_{Ar}); 135.71 (d, *J* = 6.1, C_{Ar}); 154.9 (CO)
^{31}P-NMR ($CDCl_3$, 202 MHz): δ 2.70
$[\alpha]_D^{20}$: +0.5 (c = 0.46, methanol)
MS (ESI, *m/z*): 421.9 [M(^{79}Br)-CH_2Ph]$^-$; 423.9 [M(^{81}Br)-CH_2Ph]$^-$
HPLC (method A, t_R, min): 21.92

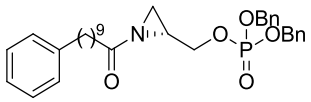
Dibenzyl [(2*R*)-1-(10-phenyldecanoyl)aziridin-2-yl]methyl phosphate, 87

a) Deprotection of amine **86**: to a solution of **86** (35 mg, 68 µmol, 1 equiv) in anhydrous dichloromethane (0.7 mL) under an argon atmosphere, TFA (0.1 mL, 1.36 mmol, 20 equiv) was added and the reaction mixture was stirred at room temperature overnight. After this time, the solvent was removed by azeotropic distillation with toluene (2x) under reduced pressure to afford the corresponding trifluoroacetate salt in quantitative yield.

b) Activation of carboxylic acid **46**: to a solution of **46** (34 mg, 0.14 mmol, 1 equiv) in anhydrous dichloromethane (2 mL) under an argon atmosphere, *N*-hydroxysuccinimide (32 mg, 0.28 mmol, 2 equiv) and EDC (39 mg, 0.21 mmol, 1.5 equiv) were added successively. The reaction was stirred at room temperature for 12 h. Then the mixture was washed with water, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the corresponding *N*-hydroxysuccinimidyl ester of **46** in quantitative yield, which was used in next step without further purification.

c) Coupling reaction: to a solution of the trifluoroacetate salt of deprotected amine **86** (35 mg, 66 µmol, 1 equiv) in anhydrous dichloromethane (4 mL) under an argon atmosphere, triethylamine (46 µL, 0.33 mmol, 5 equiv) and *N*-hydroxysuccinimidyl ester of **46** (45 mg, 0.13 mmol, 2 equiv) were added successively. The reaction was stirred at room temperature for 12 h. After this time, the mixture was washed with water, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The

residue was purified by flash chromatography (hexane/ethyl acetate, 3:2 to 1:2) to afford **87** in 48% yield.

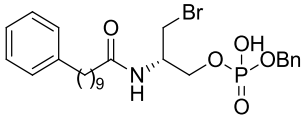


87

R_f: 0.55 (ethyl acetate)
IR (ATR): 1702 (C=O); 1261 (P=O); 1018 (P-O)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 1.25-1.32 (m, 10H, 5CH_2); 1.56-1.61 (m, 4H, 2CH_2); 2.04 (d, *J* = 2.8, 1H, ½CH_2N); 2.30-2.40 (m, 3H, CH_2CO, ½CH_2N); 2.59 (t, *J* = 7.7, 2H, CH_2Ph); 2.62-2.65 (m, 1H, CH); 3.96 (ddd, *J* = 11.2, 8.6, 6.2, 1H, ½CH_2O); 4.04 (ddd, *J* = 11.8, 7.2, 4.8, 1H, ½CH_2O); 5.02-5.09 (m, 4H, 2OCH_2Ph); 7.16-7.18 (m, 3H, 3CH_{Ar}); 7.26-7.28 (m, 2H, 2CH_{Ar}); 7.32-7.37 (m, 10H, 10CH_{Ar})
^{13}C-NMR (methanol-d_4, 175 MHz): δ 25.1, 28.4, 29.4, 29.45, 29.50, 29.54, 29.6, 31.7 (8CH_2); 34.9 (d, *J* = 8.4, CH); 36.1 (CH_2Ph); 36.6 (CH_2); 68.1 (d, *J* = 5.3, CH_2O); 69.6 (d, *J* = 6.1, OCH_2Ph); 69.7 (d, *J* = 5.4, OCH_2Ph); 125.7, 128.15, 128.17, 128.4, 128.5, 128.78, 128.82 (15CH_{Ar}); 135.7, 135.8, 143.1 (3C_{Ar}); 185.7 (CO)
$[\alpha]_D^{20}$: +6.1 (c = 0.36, methanol)
MS (MALDI, *m/z*): 564.3 $[M+H]^+$

Benzyl (2*S*)-3-bromo-2-[(10-phenyldecanoyl)amino]propyl hydrogen phosphate, 89

To a solution of **87** (4 mg, 7 µmol, 1 equiv) in anhydrous diethyl ether (1 mL) under an argon atmosphere, magnesium bromide ethyl etherate (3.7 mg, 14 µmol, 2 equiv) was added and the reaction mixture was stirred at room temperature for 4 h. After this time, the solvent was removed under reduced pressure to afford compound **89** in quantitative yield.



89

^{1}H-NMR (methanol-d_4, 300 MHz): δ 1.21-1.38 (m, 10H, 5CH_2); 1.53-1.64 (m, 4H, 2CH_2); 2.13-2.25 (m, 2H, CH_2CO); 2.58 (t, *J* = 7.6, 2H, CH_2Ph); 3.44-3.50 (m, 1H, ½CH_2Br); 3.56-3.63 (m, 1H, ½CH_2Br); 3.84-4.00 (m, 2H, CH_2OP); 4.20-4.33 (m, 1H, CH); 4.85-4.95 (m, 2H, OCH_2Ph); 7.07-7.41 (m, 10H, 10CH_{Ar})
^{31}P-NMR (methanol-d_4, 121 MHz): δ -0.71
MS (ESI, *m/z*): 552.1 $[M(^{79}Br)-H]^-$; 554.2 $[M(^{81}Br)-H]^-$
HPLC (method A, t_R, min): 9.67

4.3. Synthesis of new antagonists for the LPA_1 and LPA_2 receptors

4.3.1. General procedures

4.3.1.1. Friedel-Crafts acylation of resorcinol

a) Preparation of the aryloxyacetyl chloride: to a solution of the corresponding aryloxyacetic acid (1 equiv) in anhydrous toluene (5.5 mL/mmol) under an argon atmosphere, thionyl chloride (2.8 mL/mmol) was added and the reaction mixture was refluxed for 6 h. After this time, the excess of thionyl chloride and toluene were evaporated under reduced pressure, affording the corresponding aryloxyacetyl chloride in quantitative yield.

b) Friedel-Crafts acylation: to a cooled (0°C) stirred solution of the corresponding aryloxyacetyl chloride (1 equiv) and resorcinol (1.1 equiv) in anhydrous dichloromethane (1.5 mL/mmol) under an argon atmosphere, $BF_3 \cdot Et_2O$ (1.3 mL/mmol) was added. The reaction was stirred at 0ºC for 10 min and then at 90ºC for 4 h. The reaction vessel was then cooled in an ice bath and the mixture poured into an excess of ice water. The aqueous phase was extracted with dichloromethane, and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to yield the corresponding 2,4-dihydroxyphenyl ketone.

4.3.1.2. Synthesis of chromones by Allan-Robinson reaction

A mixture of the corresponding 2,4-dihydroxyphenyl ketone (1 equiv), freshly distilled acetic anhydride (0.6 mL/mmol), triethylamine (0.8 mL/mmol) and anhydrous sodium acetate (2.4 equiv) was stirred at 140°C for 2.5 h under an argon atmosphere. Afterward, cold water was added and the mixture was extracted with dichloromethane, washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure, affording the corresponding acetoxychromone in quantitative yield.

4.3.1.3. Hydrolysis of acetoxychromone derivatives

To a solution of the corresponding acetoxychromone (1 equiv) in the minimum amount of absolute ethanol, 36% HCl (0.6 mL/mmol) was added and the reaction was refluxed for 2 h. After cooling to room temperature, the mixture was diluted with ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure, affording the corresponding hydroxychromone in quantitative yield.

4.3.1.4. Alkylation of hydroxychromone derivatives

To a solution of the corresponding hydroxychromone (1 equiv) in anhydrous acetone (15 mL/mmol) under an argon atmosphere, K_2CO_3 (2 equiv) was added and the reaction mixture was refluxed for 30 min. Then, a solution of the appropriate alkyl 2-bromoacetate (3.5 equiv) in anhydrous acetone (1 mL/mmol) was added and the mixture was refluxed during 3 h. Then, cold water was added and acetone was removed under reduced pressure. The aqueous residue was extracted with dichloromethane, and the organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to afford the corresponding alkylated chromone.

4.3.1.5. Synthesis of pyrazole derivatives by reaction with hydrazine

A solution of the corresponding chromone or enaminone (1 equiv) in absolute ethanol (5 mL/mmol) at 40 °C under an argon atmosphere was treated with a solution of hydrazine monohydrate (65%, 0.18 mL/mmol) in absolute ethanol (1.3 mL/mmol). The reaction was refluxed for 30 min. After cooling to room temperature, the mixture was concentrated under vacuum, diluted with ethyl acetate and acidified with 1M HCl to pH 6. The aqueous phase was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to yield the corresponding pyrazole.

4.3.1.6. Williamson ether synthesis

To a solution of 2,5-dichlorophenol (1 equiv) and DBU (1.2 equiv) in anhydrous DMF (2.6 mL/mmol) under an argon atmosphere, the appropriate 2-bromoacetophenone (1.2 equiv) was added and the mixture was heated at 140°C for 30 min under MW irradiation. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography to yield the corresponding ether.

4.3.1.7. Enaminone formation

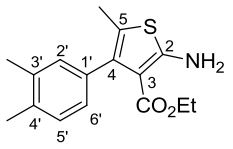
A solution of the appropriate 2-aryloxy-1-phenylethanone (1 equiv) in 1,1-dimethoxy-*N*,*N*-dimethylethylamine (4.2 equiv) was stirred at 90ºC for 2-4 h under an argon atmosphere. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure and the crude was purified by flash chromatography to afford the corresponding enaminone.

4.3.2. Synthesis of final compound **96**

Ethyl 2-amino-4-(3,4-dimethylphenyl)-5-methylthiophene-3-carboxylate, 90

To a cooled (0°C) stirred solution of titanium tetrachloride (5.07 mL, 46.23 mmol, 5 equiv) in anhydrous THF (185 mL) under an argon atmosphere, a solution of 1-(3,4-dimethylphenyl)propan-1-one (1.5 g, 9.25 mmol, 1 equiv) and ethyl 2-cyanoacetate (1.97 mL, 18.49 mmol, 2 equiv) in anhydrous THF (46 mL) was added. Then, triethylamine (2.96 mL, 21.27 mmol, 2.3 equiv) was added and the mixture was stirred at room temperature overnight. Afterward, the solvent was evaporated and the residue was dissolved in ethyl acetate and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure, affording ethyl 2-cyano-3-(3,4-dimethylphenyl)pent-2-enoate in quantitative yield.

To a solution of ethyl 2-cyano-3-(3,4-dimethylphenyl)pent-2-enoate (2.1 g, 9.15 mmol, 1 equiv) in anhydrous THF (37 mL) under an argon atmosphere, diethylamine (1.72 mL, 16.64 mmol, 1.8 equiv) and sulfur (593 mg, 18.53 mmol, 2 equiv) were added and the reaction mixture was stirred at room temperatura overnight. Afterward, the solvent was evaporated and the crude was dissolved in ethyl acetate and washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Flash chromatography of the residue (triethylamine-neutralized silica gel, hexane to hexane/ethyl acetate, 95:5) afforded pure 2-aminothiophene **90** in 32% yield.

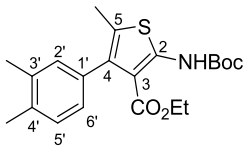


90

R_f: 0.53 (hexane/ethyl acetate, 8:2)
IR (ATR): 3394 (NH_2); 1658 (C=O); 1014 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.84 (t, *J* = 7.1, 3H, $CH_2C\underline{H}_3$); 2.05 (s, 3H, CH_3C_5); 2.26 (s, 3H, CH_3C_{Ar}); 2.28 (s, 3H, CH_3C_{Ar}); 3.95 (q, *J* = 7.1, 2H, CH_2); 5.90 (br s, 2H, NH_2); 6.88 (dd, *J* = 7.6, 1.8, 1H, $H_{6'}$); 6.93 (s, 1H, $H_{2'}$); 7.08 (d, *J* = 7.6, 1H, $H_{5'}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 13.2, 13.7 ($2CH_3$); 19.7, 19.9 ($2\underline{C}H_3C_{Ar}$); 59.2 (CH_2); 107.2, 116.9 ($2C_{Ar}$); 127.2, 128.7, 131.1 ($3CH_{Ar}$); 134.6, 135.3, 135.4, 136.5 ($4C_{Ar}$); 160.7 (C_2); 165.8 (C=O)
MS (ESI, *m/z*): 290.0 $[M+H]^+$
HPLC (method A, t_R, min): 27.16

Ethyl 2-[(*tert*-butoxycarbonyl)amino]-4-(3,4-dimethylphenyl)-5-methyl thiophene-3-carboxylate, 92

To a solution of 2-aminothiophene **90** (857 mg, 2.96 mmol, 1 equiv) in anhydrous THF (50 mL) under an argon atmosphere, DMAP (36 mg, 0.30 mmol, 0.1 equiv) and di-*tert*-butyl dicarbonate (776 mg, 3.55 mmol, 1.2 equiv) were added and the reaction mixture was stirred at room temperature overnight. Afterward, the solvent was evaporated and the residue was dissolved in ethyl acetate and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (triethylamine-neutralized silica gel, hexane to hexane/ethyl acetate, 8:2) to afford pure compound **92** in 28% yield.

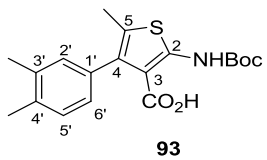


92

R_f: 0.71 (hexane/ethyl acetate, 8:2)
IR (ATR): 3284 (NH); 1720, 1663 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.81 (t, *J* = 7.1, 3H, CH_2CH_3); 1.53 (s, 9H, $3CH_3$); 2.12 (s, 3H, CH_3C_5); 2.26 (s, 3H, CH_3C_{Ar}); 2.29 (s, 3H, CH_3C_{Ar}); 3.96 (q, *J* = 7.1, 2H, CH_2); 6.86 (d, *J* = 7.7, 1H, $H_{6'}$); 6.91 (s, 1H, $H_{2'}$); 7.09 (d, *J* = 7.6, 1H, $H_{5'}$); 10.26 (s, 1H, NH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 13.0, 13.4 ($2CH_3$); 19.7, 19.9 ($2CH_3C_{Ar}$); 28.4 ($3CH_3$); 60.0 (CH_2); 82.0 ($C(CH_3)_3$); 109.7, 111.3, 124.3 ($3C_{Ar}$); 127.2, 128.8, 131.1 ($3CH_{Ar}$); 134.8, 134.9, 135.3, 135.4 ($4C_{Ar}$); 149.0 (CONH); 166.0 (CO_2)

2-[(*tert*-Butoxycarbonyl)amino]-4-(3,4-dimethylphenyl)-5-methylthiophene-3-carboxylic acid, 93

To a solution of thiophene **92** (149 mg, 0.38 mmol, 1 equiv) in a 1:1 mixture of ethanol:water (10 mL), potassium hydroxide (43 mg, 0.77 mmol, 2 equiv) was added and the reaction mixture was stirred at 60ºC overnight. After cooling to room temperature, the mixture was acidified with 1M HCl solution and extracted with ethyl acetate (2x). The combined organic phases were washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure, affording acid derivative **93** in 89% yield.

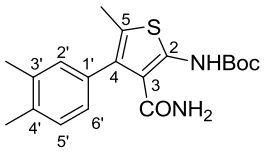


93

R_f: 0.26 (hexane/ethyl acetate, 8:2)
IR (ATR): 3305 (NH, OH); 1720, 1642 (C=O); 1018 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.55 (s, 9H, $3CH_3$); 2.10 (s, 3H, CH_3C_5); 2.28 (s, 3H, CH_3C_{Ar}); 2.30 (s, 3H, CH_3C_{Ar}); 6.96 (d, *J* = 7.7, 1H, $H_{6'}$); 6.99 (s, 1H, $H_{2'}$); 7.17 (d, *J* = 7.6, 1H, $H_{5'}$); 10.22 (s, 1H, NH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 13.0 (CH_3C_5); 19.8, 20.0 ($2CH_3C_{Ar}$); 28.4 ($3CH_3$); 77.4 ($C(CH_3)_3$); 109.7, 124.8 ($2C_{Ar}$); 127.5, 129.6, 131.2 ($3CH_{Ar}$); 133.5, 134.6, 136.0, 136.5 ($4C_{Ar}$); 151.3, 152.4 (C_2, CONH); 168.5 (CO_2H)
MS (ESI, *m/z*): 360.0 $[M-H]^-$
HPLC (method A, t_R, min): 27.10

tert-Butyl [3-carbamoyl-4-(3,4-dimethylphenyl)-5-methylthiophen-2-yl] carbamate, 94

To a solution of carboxylic acid **93** (114 mg, 0.32 mmol, 1 equiv) in anhydrous dichloromethane (0.7 mL) under an argon atmosphere, EDC·HCl (121 mg, 0.63 mmol, 2 equiv) and HOBt (85 mg, 0.63 mmol, 2 equiv) were added and the reaction mixture was stirred at room temperature for 1 h. After this time, a solution of 0.5 M NH_3 in dioxane (6.50 mL, 3.20 mmol, 10 equiv) and DIPEA (0.17 mL, 0.95 mmol, 3 equiv) were added and the mixture was stirred at room temperature overnight. Afterward, the mixture was washed sequentially with 1 M HCl, a saturated aqueous solution of $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (triethylamine-neutralized silica gel, hexane to hexane/dichloromethane, 1:1) to afford pure compound **94** in 70% yield.

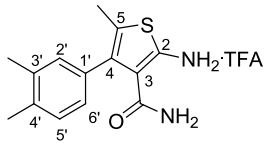


94

R_f: 0.51 (hexane/ethyl acetate, 8:2)
IR (ATR): 3459 (NH_2); 1714, 1645 (C=O); 1157 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.52 (s, 9H, $3CH_3$); 2.05 (s, 3H, CH_3C_5); 2.28 (s, 3H, CH_3C_{Ar}); 2.30 (s, 3H, CH_3C_{Ar}); 5.28 (s, 1H, ½NH_2); 5.48 (s, 1H, ½NH_2); 6.99 (d, *J* = 7.6, 1H, $H_{6'}$); 7.03 (s, 1H, $H_{2'}$); 7.20 (d, *J* = 7.6, 1H, $H_{5'}$); 11.14 (s, 1H, NH)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 12.9 (CH_3C_5); 19.7, 19.9 ($2CH_3C_{Ar}$); 28.4 ($3CH_3$); 81.6 ($C(CH_3)_3$); 112.0, 124.6 ($2C_{Ar}$); 127.6, 130.4, 131.3 ($3CH_{Ar}$); 133.3, 133.4, 137.0, 137.6 ($4C_{Ar}$); 148.3, 152.7 (C_2, CONH); 167.8 ($CONH_2$)
MS (ESI, *m/z*): 361.1 $[M+H]^+$; 261.1 $[M-CO_2tBu+2H]^+$
HPLC (method A, t_R, min): 19.67

3-Carbamoyl-4-(3,4-dimethylphenyl)-5-methylthiophen-2-aminium trifluoroacetate, 91

To a solution of *N*-Boc amine **94** (77 mg, 0.21 mmol, 1 equiv) in anhydrous dichloromethane (2.1 mL) under an argon atmosphere, TFA (0.2 mL) was added and the reaction mixture was stirred at room temperature overnight. After this time, the solvent was removed by azeotropic distillation with toluene (2x) under reduced pressure to afford trifluoroacetate salt **91** in quantitative yield.

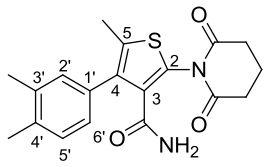


91

IR (ATR): 3382, 3358 (NH_2); 1684 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.98 (s, 3H, CH_3C_5); 2.27 (s, 3H, CH_3C_{Ar}); 2.29 (s, 3H, CH_3C_{Ar}); 5.02 (br s, 1H, ½NH_2); 5.25 (br s, 1H, ½NH_2); 6.30 (br s, 2H, NH_2); 6.99 (d, *J* = 7.7, 1H, $H_{6'}$); 7.03 (s, 1H, $H_{2'}$); 7.18 (d, *J* = 7.5, 1H, $H_{5'}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 13.0 ($\underline{C}H_3C_5$); 19.7, 19.9 (2$\underline{C}H_3C_{Ar}$); 107.7, 116.9 (2C_{Ar}); 127.5, 130.3, 131.2 (3CH_{Ar}); 134.1, 134.4, 136.7, 137.4 (4C_{Ar}); 160.4 (C_2); 168.3 ($CONH_2$)
MS (ESI, *m/z*): 261.1 $[M+H]^+$
HPLC (method A, t_R, min): 12.81

4-(3,4-Dimethylphenyl)-2-(2,6-dioxopiperidin-1-yl)-5-methylthiophene-3-carboxamide, 95

To a solution of 2-aminothiophene **91** (79 mg, 0.21 mmol, 1 equiv) in anhydrous dichloromethane (2.3 mL) under an argon atmosphere, glutaric anhydride (48 mg, 0.42 mmol, 2 equiv), DMAP (2.6 mg, 0.02 mmol, 0.1 equiv) and triethylamine (0.06 mL, 0.42 mmol, 2 equiv) were added and the reaction was refluxed for 24 h. After cooling to room temperature, the mixture was concentrated under reduced pressure, diluted with ethyl acetate and washed with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (triethylamine-neutralized silica gel, hexane/ethyl acetate, 6:4 to ethyl acetate/metanol, 95:5) affording compound **95** in 33% yield.

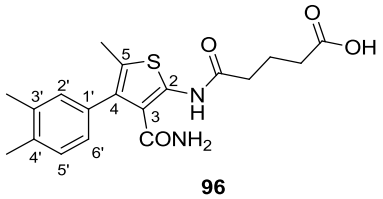


95

R_f: 0.33 (ethyl acetate)
IR (ATR): 3463 (NH_2); 1695 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.09 (m, 2H, CH_2); 2.25 (s, 3H, CH_3C_5); 2.27 (s, 3H, CH_3C_{Ar}); 2.29 (s, 3H, CH_3C_{Ar}); 2.81 (m, 4H; $2CH_2CO$); 5.19 (br s, 1H, ½NH_2); 5.39 (br s, 1H, ½NH_2); 7.06 (d, *J* = 7.7, 1H, $H_{6'}$); 7.10 (s, 1H, $H_{2'}$); 7.19 (d, *J* = 7.6, 1H, $H_{5'}$)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.1 (CH_3C_5); 16.9 (CH_2); 19.7, 19.9 ($2CH_3C_{Ar}$); 33.3 ($2CH_2CO$); 127.5, 130.3 ($2CH_{Ar}$); 130.8 (C_{Ar}); 131.2 (CH_{Ar}); 132.5, 135.2, 136.2, 136.3, 137.0, 137.5 ($6C_{Ar}$); 164.2 ($CONH_2$); 172.9 (2CON)
MS (ESI, *m/z*): 357.1 $[M+H]^+$
HPLC (method A, t_R, min): 15.56

2-[(4-Carboxybutanoyl)amino]-4-(3,4-dimethylphenyl)-5-methylthiophene-3-carboxylic acid, 96

To a solution of **95** (11.3 mg, 0.03 mmol, 1 equiv) in water (0.1 mL), triethylamine (0.01 mL, 0.10 mmol, 3.1 equiv) was added and the reaction was refluxed for 15 min. After cooling to room temperature, the mixture was treated with 1 M HCl and extracted with ethyl acetate (2x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure, affording final compound **96** in 72% yield.



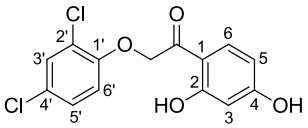
96

R_f: 0.31 (ethyl acetate)
IR (ATR): 3203 (NH, OH); 1676, 1643 (C=O)
^{1}H-NMR ($CDCl_3$, 700 MHz): δ 2.03-2.07 (m, 2H, $CH_2CH_2CO_2H$); 2.07 (s, 3H, CH_3C_5); 2.28 (s, 3H, $CH_3C_{3'}$); 2.30 (s, 3H, $CH_3C_{4'}$); 2.38 (t, *J* = 6.8, 2H, CH_2CO_2H); 2.56 (t, *J* = 7.5, 2H, $NHCOCH_2$); 5.43 (s, 1H, ½$CONH_2$); 6.53 (s, 1H, ½$CONH_2$); 6.97 (d, *J* = 7.5, 1H, $H_{6'}$); 7.01 (s, 1H, $H_{2'}$); 7.20 (d, *J* = 7.6, 1H, $H_{5'}$); 12.14 (s, 1H, NHCO)
^{13}C-NMR ($CDCl_3$, 175 MHz): δ 12.9 (CH_3C_5); 19.7 ($CH_3C_{4'}$) 20.0 ($CH_3C_{3'}$); 21.2 ($CH_2CH_2CO_2H$); 34.29 (CH_2CO_2H); 36.2 ($NHCOCH_2$);112.9 (C_3); 126.3 (C_5); 127.6 ($C_{6'}$); 130.6 ($C_{5'}$); 131.2 ($C_{2'}$); 133.0 ($C_{1'}$); 133.1 (C_4); 137.2 ($C_{4'}$); 137.8 ($C_{3'}$); 146.3 (C_2); 168.8 ($CONH_2$); 170.0 (CONH); 177.9 (CO_2H)
HRMS (ESI, *m/z*): calculated for $C_{19}H_{21}N_2O_4S$ $[M-H]^-$: 373.1227, found: 373.1195
HPLC (method A, t_R, min): 21.07

4.3.3. Synthesis of final compound **101**

2-(2,4-Dichlorophenoxy)-1-(2,4-dihydroxyphenyl)ethanone, 97

Following the general procedure 4.3.1.1., compound **97** was obtained from 2,4-dichlorophenoxyacetic acid (508 mg, 2.30 mmol) in 20% yield. Chromatography: hexane to dichloromethane.

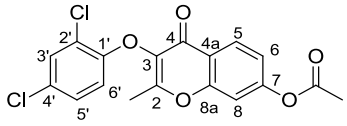


97

R_f: 0.41 (hexane/ethyl acetate, 7:3)
m.p.: 190-192 ºC
IR (ATR): 3352 (OH); 1628 (C=O); 1233 (C-O-C)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 5.45 (s, 2H, CH_2); 6.31 (d, *J* = 2.3, 1H, H_3); 6.41 (dd, *J* = 8.9, 2.3, 1H, H_5); 6.95 (d, *J* = 8.9, 1H, $H_{6'}$); 7.22 (dd, *J* = 8.8, 2.6, 1H, $H_{5'}$); 7.43 (d, *J* = 2.5, 1H, $H_{3'}$); 7.79 (d, *J* = 8.8, 1H, H_6)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 71.8 (CH_2); 103.8 (C_3); 109.6 (C_5); 112.4 (C_1); 116.1 ($C_{6'}$); 124.7, 127.3 ($C_{2'}$, $C_{4'}$); 128.8, 130.8 ($C_{3'}$, $C_{5'}$); 132.9 (C_6); 154.4 ($C_{1'}$); 166.2, 167.0 (C_2, C_4); 198.0 (C=O)
MS (ESI, *m/z*): 311.0, 313.0, 315.0 $[M-H]^-$
HPLC (method C, t_R, min): 26.82

3-(2,4-Dichlorophenoxy)-2-methyl-4-oxo-4*H*-chromen-7-yl acetate, 98

Following the general procedure 4.3.1.2., chromone **98** was obtained from 2,4-dihydroxyphenyl ketone **97** (53 mg, 0.17 mmol) in 99% yield.



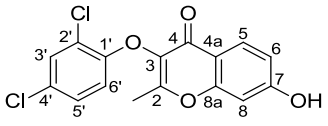
98

R_f: 0.49 (hexane/ethyl acetate, 7:3)
m.p.: 158-160 ºC
IR (ATR): 1764, 1651 (C=O); 1206 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H, $COCH_3$); 2.47 (s, 3H, C_2CH_3); 6.65 (d, *J* = 8.8, 1H, $H_{6'}$); 7.07 (dd, *J* = 8.8, 2.5, 1H, $H_{5'}$); 7.15 (dd, *J* = 8.7, 2.1, 1H, H_6); 7.32 (d, *J* = 2.1, 1H, H_8); 7.44 (d, *J* = 2.5, 1H, $H_{3'}$); 8.21 (d, *J* = 8.7, 1H, H_5)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 15.9 ($C_2\underline{C}H_3$); 21.3 ($CO\underline{C}H_3$); 111.1 (C_8); 115.6 ($C_{6'}$); 119.6 (C_6); 122.1 (C_{4a}); 123.7 ($C_{2'}$); 127.6 (C_5); 127.7 ($C_{5'}$); 127.9 ($C_{4'}$); 130.5 ($C_{3'}$); 137.0 (C_3); 151.6 ($C_{1'}$); 154.7 (C_7); 156.0 (C_{8a}); 161.0 (C_2); 168.7 (C=O); 171.3 (C_4)

MS (ESI, *m/z*): 379.0, 381.0, 383.0 $[M+H]^+$
HPLC (method C, t_R, min): 27.32

3-(2,4-Dichlorophenoxy)-7-hydroxy-2-methyl-4*H*-chromen-4-one, 99

Following the general procedure 4.3.1.3., compound **99** was obtained from acetoxychromone **98** (61 mg, 0.16 mmol) in 99% yield.

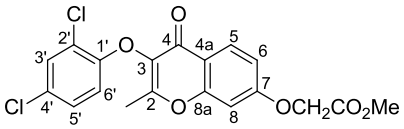


99

R_f: 0.29 (hexane/ethyl acetate, 7:3)
m.p.: 190-192 ºC
IR (ATR): 3100 (OH); 1595 (C=O); 1268 (C-O-C)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.42 (s, 3H, CH_3); 6.79 (d, *J* = 8.8, 1H, $H_{6'}$); 6.90 (d, *J* = 2.2, 1H, H_8); 6.94 (dd, *J* = 8.8, 2.2, 1H, H_6); 7.16 (dd, *J* = 8.8, 2.5, 1H, $H_{5'}$); 7.51 (d, *J* = 2.5, 1H, $H_{3'}$); 7.96 (d, *J* = 8.8, 1H, H_5)
^{13}C-NMR (methanol-d_4, 176 MHz): δ 15.6 (CH_3); 103.4 (C_8); 116.5 (C_6); 116.7($C_{6'}$); 117.7 (C_{4a}); 124.2 ($C_{2'}$/$C_{4'}$); 128.1 (C_5); 128.4 ($C_{2'}$/$C_{4'}$); 128.9 ($C_{5'}$); 131.1 ($C_{3'}$); 136.9 (C_3); 153.2 ($C_{1'}$); 159.2 (C_7/C_{8a}); 162.6 (C_2); 165.1 (C_7/C_{8a}); 173.5 (C_4)
MS (ESI, *m/z*): 334.9, 336.9, 338.9 $[M-H]^-$
HPLC (method C, t_R, min): 25.95

Methyl {[3-(2,4-dichlorophenoxy)-2-methyl-4-oxo-4*H*-chromen-7-yl]oxy}acetate, 100

Following the general procedure 4.3.1.4., compound **100** was obtained from hydroxychromone **99** (81 mg, 0.24 mmol) and methyl 2-bromoacetate (0.09 mL, 0.84 mmol) in 51% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.



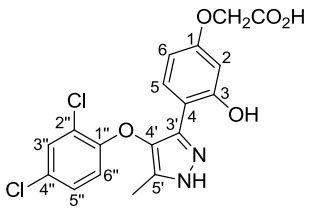
100

R_f: 0.33 (hexane/ethyl acetate, 7:3)
m.p.: 176-178 ºC
IR (ATR): 1760 (C=O); 1640 (C=O); 1256 (C-O-C)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.44 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3); 4.75 (s, 2H, CH_2); 6.65 (d, *J* = 8.8, 1H, $H_{6'}$); 6.86 (d, *J* = 2.4, 1H, H_8); 7.02 (dd, *J* = 8.9, 2.4, 1H, H_6); 7.06 (dd, *J* = 8.8, 2.5, 1H, $H_{5'}$); 7.43 (d, *J* = 2.5, 1H, $H_{3'}$); 8.13 (d, *J* = 8.9, 1H, H_5)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 15.8 (CH_3); 52.7 (OCH_3); 65.5 (CH_2); 101.5 (C_8); 114.6 (C_6); 115.6 ($C_{6'}$); 118.9 (C_{4a}); 123.6 ($C_{2'}$/$C_{4'}$); 127.67 ($C_{5'}$); 127.73 ($C_{2'}$/$C_{4'}$); 128.0 (C_5); 130.5 ($C_{3'}$); 136.8 (C_3); 151.8 ($C_{1'}$); 157.1 (C_{8a}); 160.2 (C_2); 162.2 (C_7); 168.4 (C=O); 171.4 (C_4)
MS (ESI, *m/z*): 409.0, 411.0, 413.0 $[M+H]^+$
HPLC (method C, t_R, min): 26.08

{4-[4-(2,4-Dichlorophenoxy)-5-methyl-1*H*-pyrazol-3-yl]-3-hydroxyphenoxy} acetic acid, 101

Following the general procedure 4.3.1.5., pyrazole **101** was obtained from chromone **100** (45 mg, 0.11 mmol) in 99% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.



101

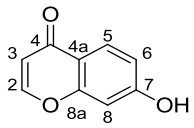
R_f: 0.93 (dichloromethane/methanol/acetic acid, 7:3:0.01)
m.p.: >196 ºC dec
IR (ATR): 3413 (OH, NH); 1619 (C=O); 1182 (C-O-C)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 2.13 (s, 3H, CH_3); 4.52 (s, 2H, CH_2); 6.36 (dd, *J* = 8.7, 2.5, 1H, H_6); 6.47 (d, *J* = 2.5, 1H, H_2); 6.66 (d, *J* = 8.9, 1H, $H_{6''}$); 7.12 (dd, *J* = 8.9, 2.5, 1H, $H_{5''}$); 7.48-7.51 (m, 2H, H_5, $H_{3''}$)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 8.7 (CH_3); 66.5 (CH_2); 103.5 (C_2); 107.1 (C_6); 110.5 (C_4); 116.8 ($C_{6''}$); 124.1, 128.3 ($C_{2''}$, $C_{4''}$); 128.6 (C_5); 129.1 ($C_{5''}$); 131.1 ($C_{3''}$); 133.2, 135.2 ($C_{4'}$, $C_{5'}$); 140.2 ($C_{3'}$); 153.9 ($C_{1''}$); 158.1 (C_3); 160.4 (C_1); 173.8 (C=O)
HRMS (ESI, *m/z*): calculated for $C_{18}H_{13}Cl_2N_2O_5$ $(M)^-$: 407.0207, found: 407.0192
HPLC (method C, t_R, min): 25.45

4.3.4. Synthesis of final compound **104**

7-Hydroxy-4*H*-chromen-4-one, 102

A solution of 1-(2,4-dihydroxyphenyl)ethan-1-one (500 mg, 3.29 mmol, 1 equiv) in triethyl orthoformate (2.9 mL, 17.4 mmol, 5.3 equiv) was treated with 70% perchloric acid (0.3 mL, 3.83 mmol, 1.2 equiv). The resulting warm and thick dark solution was stirred until it cooled to room temperature (about 1 h). Anhydrous diethyl ether (12.5 mL) was added to precipitate out the brown-colored intermediate of the oxonium perchlorate salt. The salt was taken in water and hydrolyzed by heating to reflux for 5 min. The reaction mixture was then allowed to cool at room temperature for 12 h. A

dark brown solid precipitated out, which was filtered, dried under vacuum, and purified by flash chromatography (hexane/ethyl acetate, 1:1 to ethyl acetate) to yield pure compound **102** in 47% yield.

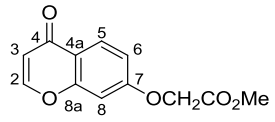


102

R_f: 0.56 (hexane/ethyl acetate, 2:8)
IR (ATR): 3526 (OH); 1630 (C=O)
^{1}H-NMR (acetone-d_6, 300 MHz): δ 7.27 (d, *J* = 5.6, 1H, H_3); 7.34 (d, *J* = 2.2, 1H, H_8); 7.40 (dd, *J* = 9.0, 2.3, 1H, H_6); 8.33 (d, *J* = 9.0, 1H, H_5); 8.98 (d, *J* = 5.6, 1H, H_2)
^{13}C-NMR (acetone-d_6, 75 MHz): δ 103.6 (C_8); 108.5 (C_3); 120.2 (C_6); 120.3 (C_{4a}); 128.3 (C_5); 154.5 (C_2); 161.6 (C_{8a}); 164.4 (C_7); 167.7 (C_4)
MS (ESI, *m/z*): 161.1 $[M-H]^-$
HPLC (method C, t_R, min): 11.51

Methyl [(4-oxo-4*H*-chromen-7-yl)oxy]acetate, 103

Following the general procedure 4.3.1.4., compound **103** was obtained from chromone **102** (147 mg, 0.91 mmol) and methyl 2-bromoacetate (0.35 mL, 3.19 mmol) in 24% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.

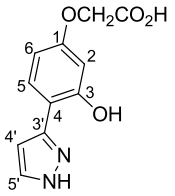


103

R_f: 0.21 (hexane/ethyl acetate, 7:3)
m.p.: 146-148 ºC
IR (ATR): 1756 (C=O); 1648 (C=O); 1214 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 3.83 (s, 3H, CH_3); 4.73 (s, 2H, CH_2); 6.28 (d, *J* = 6.0, 1H, H_3); 6.82 (d, *J* = 2.4, 1H, H_8); 7.00 (dd, *J* = 8.9, 2.4, 1H, H_6); 7.77 (d, *J* = 6.0, 1H, H_2); 8.13 (d, *J* = 8.9, 1H, H_5)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 52.7 (CH_3); 65.4 (CH_2); 101.7 (C_8); 113.2, 114.5 (C_3, C_6); 119.7 (C_{4a}); 127.8 (C_5); 155.1 (C_2); 158.1 (C_{8a}); 162.1 (C_7); 168.4 (C=O); 177.0 (C_4)
MS (ESI, *m/z*): 235.1 $[M+H]^+$
HPLC (method C, t_R, min): 12.69

[3-Hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]acetic acid, 104

Following the general procedure 4.3.1.5., pyrazole **104** was obtained from chromone **103** (29 mg, 0.12 mmol) in 98% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.



104

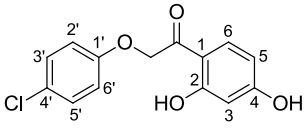
R_f: 0.79 (dichloromethane/methanol, 7:3)
m.p.: 92-94 ºC
IR (ATR): 3404 (OH, NH); 1623 (C=O)
^{1}H-NMR (DMSO-d_6, 700 MHz): δ 4.35 (s, 2H, CH_2); 6.41-6.42 (m, 2H, H_2, H_6); 6.71 (br s, 1H, $H_{4'}$); 7.56 (d, *J* = 8.7, 1H, H_5); 7.80 (br s, 1H, $H_{5'}$); 11.04 (br s, 1H, OH); 13.16 (br s, 1H, NH)
^{13}C-NMR (DMSO-d_6, 175 MHz): δ 66.6 (CH_2); 101.3 ($C_{4'}$); 102.0 (C_2); 106.3 (C_6); 110.0 (C_4); 127.4 (C_5); 130.0 ($C_{5'}$); 150.0 ($C_{3'}$); 156.2 (C_3); 159.1 (C_1); 171.5 (C=O)
HRMS (ESI, *m/z*): calculated for $C_{11}H_9N_2O_4$ $[M-H]^-$: 233.0568, found: 233.0559
HPLC (method C, t_R, min): 11.02

4.3.5. Synthesis of final compounds **105**-**107**

4.3.5.1. Synthesis of 2,4-dihydroxyphenyl ketones **108-110**

2-(4-Chlorophenoxy)-1-(2,4-dihydroxyphenyl)ethanone, 108

Following the general procedure 4.3.1.1., aryl ketone **108** was obtained from 4-chlorophenoxyacetic acid (500 mg, 2.68 mmol) in 20% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.



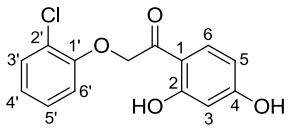
108

R_f: 0.71 (hexane/ethyl acetate, 7:3)
m.p.: 181-183 ºC
IR (ATR): 3321 (OH); 1629 (C=O); 1233 (C-O-C)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 5.33 (s, 2H, CH_2); 6.31 (d, *J* = 2.3, 1H, H_3); 6.41 (dd, *J* = 8.9, 2.4, 1H, H_5); 6.93-6.97 (m, 2H, $H_{2'}$, $H_{6'}$); 7.23-7.28 (m, 2H, $H_{3'}$,$H_{5'}$); 7.78 (d, *J* = 8.9, 1H, H_6)

^{13}C-NMR (methanol-d_4, 75 MHz): δ 70.9 (CH_2); 103.8 (C_3); 109.6 (C_5); 112.4 (C_1); 117.3 ($C_{2'}$, $C_{6'}$); 127.2 ($C_{4'}$); 130.3 ($C_{3'}$, $C_{5'}$); 132.8 (C_6); 158.5 ($C_{1'}$); 166.2, 167.0 (C_2, C_4); 198.7 (C=O)
MS (ESI, *m/z*): 277.0 [M(^{35}Cl)-H]$^-$, 279.0 [M(^{37}Cl)-H]$^-$
HPLC (method C, t_R, min): 27.20

2-(2-Chlorophenoxy)-1-(2,4-dihydroxyphenyl)ethanone, 109

Following the general procedure 4.3.1.1., aryl ketone **109** was obtained from 2-chlorophenoxyacetic acid (50 mg, 2.68 mmol) in 20% yield. Chromatography: hexane/dichloromethane, 3:7 to dichloromethane.

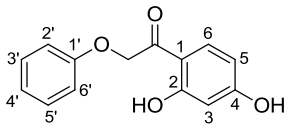


109

R_f: 0.39 (hexane/ethyl acetate, 7:3)
m.p.: 161-163 ºC
IR (ATR): 3343 (OH); 1630 (C=O); 1233 (C-O-C)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 5.37 (s, 2H, CH_2); 6.30 (d, *J* = 2.3, 1H, H_3); 6.39 (dd, *J* = 8.9, 2.3, 1H, H_5); 6.88-6.94 (m, 2H, $H_{6'}$, $H_{4'}$/$H_{5'}$); 7.14-7.20 (m, 1H, $H_{4'}$/$H_{5'}$); 7.35 (d, *J* = 8.1, 1H, $H_{3'}$); 7.77 (d, *J* = 8.9, 1H, H_6)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 71.5 (CH_2); 103.7 (C_3); 109.5 (C_5); 112.4 (C_1); 115.1, 123.1 (2CH_{Ar}); 123.8 ($C_{2'}$); 128.9, 131.3 (2CH_{Ar}); 132.9 (C_6); 155.2 ($C_{1'}$); 166.2, 166.8 (C_2, C_4); 198.4 (C=O)
MS (ESI, *m/z*): 277.0 [M(^{35}Cl)-H]$^-$, 279.0 [M(^{37}Cl)-H]$^-$
HPLC (method C, t_R, min): 25.00

1-(2,4-Dihydroxyphenyl)-2-phenoxyethanone, 110

Following the general procedure 4.3.1.1., aryl ketone **110** was obtained from phenoxyacetic acid (500 mg, 3.29 mmol) in 10% yield. Chromatography: hexane/dichloromethane, 3:7 to dichloromethane.



110

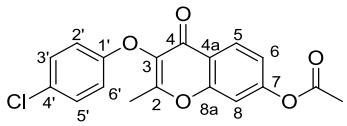
R_f: 0.44 (hexane/ethyl acetate, 7:3)
m.p.: 191-193 ºC
IR (ATR): 3316 (OH); 1628 (CO); 1231 (C-O-C)

^{1}H-NMR (methanol-d_4, 300 MHz): δ 5.31 (s, 2H, CH_2); 6.31 (d, J = 2.2, 1H, H_3); 6.41 (dd, J = 8.8, 2.2, 1H, H_5); 6.93-7.00 (m, 3H, $3H_{Ar}$); 7.25-7.30 (m, 2H, $2H_{Ar}$); 7.80 (d, J = 8.9, 1H, H_6)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 70.7 (CH_2); 103.8 (C_3); 109.6 (C_5); 112.4 (C_1); 115.8 ($2CH_{Ar}$); 122.4 (CH_{Ar}); 130.5 ($2CH_{Ar}$); 132.9 (C_6); 159.6 ($C_{1'}$); 166.3, 166.9 (C_2, C_4); 199.3 (C=O)
MS (ESI, *m/z*): 243.1 $[M-H]^-$
HPLC (method C, t_R, min): 23.38

4.3.5.2. Synthesis of acetoxychromenones **111-113**

3-(4-Chlorophenoxy)-2-methyl-4-oxo-4*H*-chromen-7-yl acetate, 111

Following the general procedure 4.3.1.2., chromone **111** was obtained from 2,4-dihydroxyphenyl ketone **108** (60 mg, 0.22 mmol) in 99% yield.

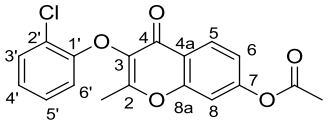


111

R_f: 0.81 (hexane/ethyl acetate, 7:3)
m.p.: 134-136 ºC
IR (ATR): 1769 (C=O); 1617 (C=O); 1237 (C-O-C)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 2.34 (s, 3H, $COCH_3$); 2.45 (s, 3H, C_2CH_3); 6.94-6.97 (m, 2H, $H_{2'}$, $H_{6'}$); 7.26-7.29 (m, 3H, $H_{3'}$, $H_{5'}$, H_6); 7.49 (d, J = 2.1, 1H, H_8); 8.15 (d, J = 8.7, 1H, H_5)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 15.8 ($C_2\underline{C}H_3$); 20.9 ($CO\underline{C}H_3$); 112.5 (C_8); 117.5 ($C_{2'}$, $C_{6'}$); 121.1 (C_6); 122.8 (C_{4a}); 127.8 (C_5); 128.4 ($C_{4'}$); 130.6 ($C_{3'}$, $C_{5'}$); 137.5 (C_3); 156.6, 157.3 (C_7, C_{8a}); 157.6 ($C_{1'}$); 163.9 (C_2); 170.1 (C=O); 173.9 (C_4)
MS (ESI, *m/z*): 345.0 $[M(^{35}Cl)+H]^+$, 347.1 $[M(^{37}Cl)+H]^+$
HPLC (method C, t_R, min): 28.23

3-(2-Chlorophenoxy)-2-methyl-4-oxo-4*H*-chromen-7-yl acetate, 112

Following the general procedure 4.3.1.2., chromone **112** was obtained from 2,4-dihydroxyphenyl ketone **109** (43 mg, 1.15 mmol) in 99% yield.



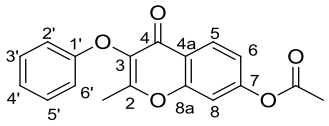
112

R_f: 0.46 (hexane/ethyl acetate, 7:3)
m.p.: 161-163 ºC

IR (ATR): 1647 (C=O); 1257 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.36 (s, 3H, $COCH_3$); 2.46 (s, 3H, C_2CH_3); 6.70 (dd, *J* = 8.2, 1.3, 1H, $H_{6'}$); 6.97 (dt, *J* = 7.7, 1.4, 1H, $H_{4'}/H_{5'}$); 7.07-7.16 (m, 2H, $H_{4'}/H_{5'}$, H_6); 7.32 (d, *J* = 2.0, 1H, H_8); 7.43 (dd, *J* = 7.9, 1.5, 1H, $H_{3'}$); 8.22 (d, *J* = 8.7, 1H, H_5)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 15.9 ($C_2\underline{C}H_3$); 21.3 ($CO\underline{C}H_3$); 111.1 (C_8); 114.6, 119.5 (C_6, $C_{6'}$); 122.1, 122.7 ($C_{2'}$, C_{4a}); 123.4, 127.6 ($2CH_{Ar}$); 127.7 (C_5); 130.8 (CH_{Ar}); 137.0 (C_3); 152.8, 154.6 (C_7, C_{8a}); 156.0 ($C_{1'}$); 161.0 (C_2); 168.7 (C=O); 171.6 (C_4)
MS (ESI, *m/z*): 345.1 [M(^{35}Cl)+H]$^+$, 347.0 [M(^{37}Cl)+H]$^+$
HPLC (method C, t_R, min): 27.87

2-Methyl-4-oxo-3-phenoxy-4*H*-chromen-7-yl acetate, 113

Following the general procedure 4.3.1.2., chromone **113** was obtained from 2,4-dihydroxyphenyl ketone **110** (35 mg, 0.14 mmol) in 81% yield.



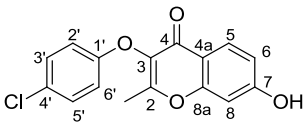
113

R_f: 0.58 (hexane/ethyl acetate, 7:3)
IR (ATR): 1768 (C=O); 1618 (C=O); 1195 (C-O-C)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.35 (s, 3H, $COCH_3$); 2.44 (s, 3H, C_2CH_3); 6.94-6.96 (m, 2H, $H_{2'}$, $H_{6'}$); 7.02 (tt, *J* = 7.4, 0.9, 1H, $H_{4'}$); 7.26-7.30 (m, 3H, H_6, $H_{3'}$, $H_{5'}$); 7.49 (d, *J* = 2.1, 1H, H_8); 8.16 (d, *J* = 8.7, 1H, H_5)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 15.8 ($C_2\underline{C}H_3$); 20.9 ($CO\underline{C}H_3$); 112.4 (C_8); 115.8 ($C_{2'}$, $C_{6'}$); 121.1 (C_6); 122.8 (C_{4a}); 123.5 ($C_{4'}$); 127.8 (C_5); 130.7 ($C_{3'}$, $C_{5'}$); 137.6 (C_3); 156.6, 157.6 (C_7, C_{8a}); 158.6 ($C_{1'}$); 163.9 (C_2); 170.1 (C=O); 174.2 (C_4)
MS (ESI, *m/z*): 311.1 [M+H]$^+$
HPLC (method C, t_R, min): 25.30

4.3.5.3. Synthesis of hydroxychromones **114**-**116**

3-(4-Chlorophenoxy)-7-hydroxy-2-methyl-4*H*-chromen-4-one, 114

Following the general procedure 4.3.1.3., hydroxychromone **114** was obtained from acetoxychromone **111** (61 mg, 0.18 mmol) in 99% yield.



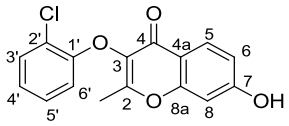
114

R_f: 0.43 (hexane/ethyl acetate, 7:3)
m.p.: >260 ºC dec
IR (ATR): 3393 (OH); 1651 (C=O)

^{1}H-NMR (methanol-d_4, 500 MHz): δ 2.33 (s, 3H, CH_3); 6.52 (d, J = 2.2, 1H, H_8); 6.69 (dd, J = 8.9, 2.2, 1H, H_6); 6.90-6.93 (m, 2H, $H_{2'}$, $H_{6'}$); 7.24-7.27 (m, 2H, $H_{3'}$, $H_{5'}$); 7.77 (d, J = 8.9, 1H, H_5)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 15.5 (CH_3); 104.3 (C_8); 116.0 (C_{4a}); 117.4 ($C_{2'}$, $C_{6'}$); 121.5 (C_6); 126.8 (C_5); 127.9 ($C_{4'}$); 130.4 ($C_{3'}$, $C_{5'}$), 136.2 (C_3); 157.8, 160.7, 160.8, 161.5 ($C_{1'}$, C_2, C_7, C_{8a}); 174.2 (C_4)
MS (ESI, *m/z*): 301.0 [M(^{35}Cl)-H]$^-$, 303.0 [M(^{37}Cl)-H]$^-$
HPLC (method C, t_R, min): 26.85

3-(2-Chlorophenoxy)-7-hydroxy-2-methyl-4*H*-chromen-4-one, 115

Following the general procedure 4.3.1.3., hydroxychromone **115** was obtained from acetoxychromone **112** (35 mg, 0.10 mmol) in 75% yield.

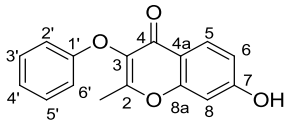


115

R_f: 0.32 (hexane/ethyl acetate, 7:3)
m.p.: 253-255 ºC
IR (ATR): 3396 (OH); 1652 (C=O)
^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.35 (s, 3H, CH_3); 6.88 (dd, J = 8.3, 1.3, 1H, $H_{6'}$); 6.90 (d, J = 2.1, 1H, H_8); 6.94 (dd, J = 8.7, 2.2, 1H, H_6); 7.03 (dt, J = 7.7, 1.3, 1H, $H_{4'}$/$H_{5'}$); 7.17 (m, 1H, $H_{4'}$/$H_{5'}$); 7.51 (dd, J = 7.9, 1.5, 1H, $H_{3'}$); 7.86 (d, J = 8.7, 1H, H_5); 10.9 (br s, 1H, OH)
^{13}C-NMR (DMSO-d_6, 125 MHz): δ 15.3 (CH_3); 102.2 (C_8); 114.8 ($C_{6'}$); 115.0 (C_6); 116.3, 120.9 ($C_{2'}$, C_{4a}); 123.1 ($C_{4'}$/$C_{5'}$); 126.9 (C_5); 128.2 ($C_{4'}$/$C_{5'}$); 130.2 ($C_{3'}$); 135.1 (C_3); 152.4, 156.9, 160.0, 162.7 ($C_{1'}$, C_2, C_{8a}, C_7); 170.4 (C_4)
MS (ESI, *m/z*): 301.0 [M(^{35}Cl)-H]$^-$, 303.0 [M(^{37}Cl)-H]$^-$
HPLC (method C, t_R, min): 25.76

7-Hydroxy-2-methyl-3-phenoxy-4*H*-chromen-4-one, 116

Following the general procedure 4.3.1.3., hydroxychromone **116** was obtained from acetoxychromone **113** (46 mg, 0.15 mmol) in 99% yield.



116

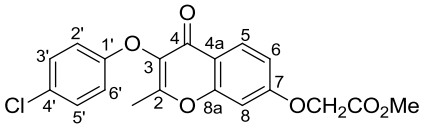
R_f: 0.29 (hexane/ethyl acetate, 7:3)
m.p.: 250-252 ºC

IR (ATR): 3229 (OH); 1594 (C=O); 1249 (C-O-C)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 2.39 (s, 3H, CH_3); 6.89-6.95 (m, 4H, H_6, H_8, $H_{2'}$, $H_{6'}$); 7.01 (t, J = 7.4, 1H, $H_{4'}$); 7.26-7.30 (m, 2H, $H_{3'}$, $H_{5'}$); 7.97 (d, J = 8.8, 1H, H_5)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 15.7 (CH_3); 103.3 (C_8); 115.8 ($C_{2'}$, $C_{6'}$); 116.4 (C_6); 117.8 (C_{4a}); 123.3 ($C_{4'}$); 128.1 (C_5); 130.7 ($C_{3'}$, $C_{5'}$); 137.0 (C_3); 158.7, 159.1, 162.6, 164.7 ($C_{1'}$, C_2, C_7, C_{8a}); 174.5 (C_4)
MS (ESI, *m/z*): 267.1 $[M-H]^-$
HPLC (method C, t_R, min): 23.82

4.3.5.4. Synthesis of chromones **117**-**119**

Methyl {[3-(4-chlorophenoxy)-2-methyl-4-oxo-4*H*-chromen-7-yl]oxy}acetate, 117

Following the general procedure 4.3.1.4., compound **117** was obtained from hydroxychromone **114** (82 mg, 0.27 mmol) and methyl 2-bromoacetate (0.10 mL, 0.95 mmol) in 50% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.

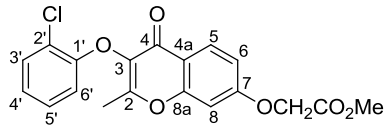


117

R_f: 0.48 (hexane/ethyl acetate, 7:3)
m.p.: 147-149 ºC
IR (ATR): 1761 (C=O); 1649 (C=O); 1237 (C-O-C)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 2.42 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 4.92 (s, 2H, CH_2); 6.92-6.95 (m, 2H, $H_{2'}$, $H_{6'}$); 7.12-7.14 (m, 2H, H_6, H_8); 7.26-7.29 (m, 2H, $H_{3'}$, $H_{5'}$); 8.06 (d, J = 9.3, 1H, H_5)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 15.7 (CH_3); 52.8 (OCH_3); 66.3 (CH_2); 102.7 (C_8); 116.1 (C_6); 117.5 ($C_{2'}$, $C_{6'}$); 119.4 (C_{4a}); 128.0 (C_5); 128.3 ($C_{4'}$); 130.5 ($C_{3'}$, $C_{5'}$); 137.3 (C_3); 157.4 ($C_{1'}$); 158.8 (C_{8a}); 163.2 (C_2); 164.3 (C_7); 170.3 (C=O); 174.0 (C_4)
MS (ESI, *m/z*): 375.1 $[M(^{35}Cl)+H]^+$, 377.0 $[M(^{37}Cl)+H]^+$
HPLC (method C, t_R, min): 27.19

Methyl {[3-(2-chlorophenoxy)-2-methyl-4-oxo-4*H*-chromen-7-yl]oxy}acetate, 118

Following the general procedure 4.3.1.4., compound **118** was obtained from hydroxychromone **115** (30 mg, 0.10 mmol) and methyl 2-bromoacetate (0.04 mL, 0.35 mmol) in 62% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.

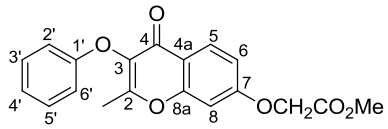


118

R_f: 0.42 (hexane/ethyl acetate, 7:3)
m.p.: 204-206 ºC
IR (ATR): 1651 (C=O)
^{1}H-NMR (DMSO-d_6, 700 MHz): δ 2.38 (s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 5.03 (s, 2H, CH_2); 6.93 (dd, *J* = 8.3, 1.3, 1H, $H_{6'}$); 7.04 (dt, *J* = 7.9, 1.3, 1H, $H_{4'}$); 7.12 (dd, *J* = 8.9, 2.4, 1H, H_6); 7.16-7.18 (m, 1H, $H_{5'}$); 7.26 (d, *J* = 2.4, 1H, H_8); 7.52 (dd, *J* = 8.0, 1.5, 1H, $H_{3'}$); 7.93 (d, *J* = 8.9, 1H, H_5)
^{13}C-NMR (DMSO-d_6, 175 MHz): δ 15.3 (CH_3); 52.0 (OCH_3); 65.0 (CH_2); 101.6 (C_8); 114.87, 114.88 (C_6, $C_{6'}$); 117.9 (C_{4a}); 121.0 ($C_{2'}$); 123.2 ($C_{4'}$); 126.6 (C_5); 128.2 ($C_{5'}$); 130.3 ($C_{3'}$); 135.4 (C_3); 152.4 ($C_{1'}$); 156.7 (C_{8a}); 160.6 (C_2); 162.1 (C_7); 168.6 (C=O); 170.4 (C_4)
MS (ESI, *m/z*): 375.0 [M(^{35}Cl)+H]$^+$, 377.0 [M(^{37}Cl)+H]$^+$
HPLC (method C, t_R, min): 26.04

Methyl [(2-methyl-4-oxo-3-phenoxy-4*H*-chromen-7-yl)oxy]acetate, 119

Following the general procedure 4.3.1.4., compound **119** was obtained from hydroxychromone **116** (33 mg, 0.12 mmol) and methyl 2-bromoacetate (0.05 mL, 0.42 mmol) in 60% yield. Chromatography: hexane to hexane/ethyl acetate, 7:3.



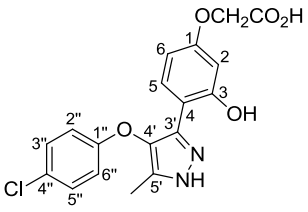
119

R_f: 0.38 (hexane/ethyl acetate, 7:3)
m.p.: 169-171 ºC
IR (ATR): 1760 (C=O); 1650(C=O); 1234 (C-O-C)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.42 (s, 3H, CH_3); 3.82 (s, 3H, OCH_3); 4.92 (s, 2H, CH_2); 6.94 (d, *J* = 8.6, 2H, $H_{2'}$, $H_{6'}$); 7.02 (t, *J* = 7.4, 1H, $H_{4'}$); 7.12-7.14 (m, 2H, H_6, H_8); 7.27-7.30 (m, 2H, $H_{3'}$, $H_{5'}$); 8.06 (d, *J* = 9.2, 1H, H_5)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 15.7 (CH_3); 52.8 (OCH_3); 66.3 (CH_2); 102.7 (C_8); 115.8 ($C_{2'}$, $C_{6'}$); 116.1 (C_6); 119.4 (C_{4a}); 123.4 ($C_{4'}$); 128.0 (C_5); 130.7 ($C_{3'}$, $C_{5'}$); 137.3 (C_3); 158.7 ($C_{1'}$); 158.8 (C_{8a}); 163.1 (C_2); 164.2 (C_7); 170.3 (C=O); 174.3 (C_4)
MS (ESI, *m/z*): 341.1 [M+H]$^+$
HPLC (method C, t_R, min): 24.50

4.3.5.5. Synthesis of pyrazoles **105**-**107**

{4-[4-(4-Chlorophenoxy)-5-methyl-1*H*-pyrazol-3-yl]-3-hydroxyphenoxy}acetic acid, 105

Following the general procedure 4.3.1.5., pyrazole **105** was obtained from chromone **117** (14 mg, 0.04 mmol) in 99% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.

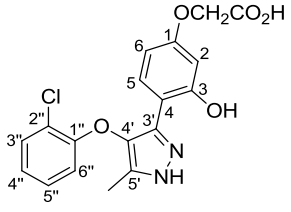


105

R_f: 0.85 (dichloromethane/methanol, 7:3)
m.p.: 121-123 ºC
IR (ATR): 1627 (C=O)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.24 (s, 3H, CH_3); 4.71 (s, 2H, CH_2); 6.44 (dd, *J* = 8.9, 2.5, 1H, H_6); 6.53 (d, *J* = 2.5, 1H, H_2); 6.97-6.99 (m, 2H, $H_{2''}$, $H_{6''}$); 7.31-7.33 (m, 2H, $H_{3''}$, $H_{5''}$); 7.64 (d, *J* = 8.8, 1H, H_5)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 8.5 (CH_3); 65.9 (CH_2); 103.3 (C_2); 107.3 (C_6); 108.2 (C_4); 117.7 ($C_{2''}$, $C_{6''}$); 128.9 ($C_{4''}$); 130.3 (C_5); 130.9 ($C_{3''}$, $C_{5''}$); 134.0, 137.5 ($C_{4'}$, $C_{5'}$); 138.3 ($C_{3'}$/$C_{4'}$); 157.5 ($C_{3'}$/$C_{4'}$); 157.6 ($C_{1''}$); 158.1 (C_3); 161.6 (C_1); 171.0 (C=O)
HRMS (ESI, *m/z*): calculated for $C_{18}H_{14}{}^{35}ClN_2O_5$ $[M-H]^-$: 373.0597, found: 373.0577; calculated for $C_{18}H_{14}{}^{37}ClN_2O_5$ $[M-H]^-$: 375.0567, found: 375.0546
HPLC (method C, t_R, min): 23.84

{4-[4-(2-Chlorophenoxy)-5-methyl-1*H*-pyrazol-3-yl]-3-hydroxyphenoxy}acetic acid, 106

Following the general procedure 4.3.1.5, pyrazole **106** was obtained from chromone **118** (19 mg, 0.05 mmol) in 99% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.



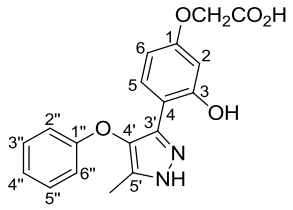
106

R_f: 0.95 (dichloromethane/methanol, 7:3)
m.p.: 149-151 ºC

IR (ATR): 3365 (OH); 1629 (C=O)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.13 (s, 3H, CH_3); 4.42 (s, 2H, CH_2); 6.33 (dd, *J* = 8.8, 2.4, 1H, H_6); 6.46 (d, *J* = 2.4, 1H, H_2); 6.67 (dd, *J* = 8.3, 1.3, 1H, $H_{6''}$); 6.97 (dt, *J* = 7.8, 1.3, 1H, $H_{4''}$); 7.16-7.07 (m, 1H, $H_{5''}$); 7.46 (dd, *J* = 8.0, 1.5, 1H, $H_{3''}$); 7.54 (d, *J* = 8.8, 1H, H_5)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 8.6 (CH_3); 67.5 (CH_2); 103.4 (C_2); 107.0 (C_6); 110.5 (C_4); 115.7 ($C_{6''}$); 123.2 ($C_{2''}$); 124.1 ($C_{4''}$); 128.5 (C_5); 129.1 ($C_{5''}$); 131.6 ($C_{3''}$); 133.2 ($C_{5'}$); 154.9 ($C_{1''}$); 158.0 (C_3); 160.6 (C_1); 176.2 (C=O); $C_{3'}$ and $C_{4'}$ not observed
HRMS (ESI, *m/z*): calculated for $C_{18}H_{14}{}^{35}ClN_2O_5$ $[M-H]^-$: 373.0597, found: 373.0574; calculated for $C_{18}H_{14}{}^{37}ClN_2O_5$ $[M-H]^-$: 375.0567, found: 375.0546
HPLC (method C, t_R, min): 22.74

[3-Hydroxy-4-(5-methyl-4-phenoxy-1*H*-pyrazol-3-yl)phenoxy]acetic acid, 107

Following the general procedure 4.3.1.5., pyrazole **107** was obtained from chromone **119** (10 mg, 0.03 mmol) in 99% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.



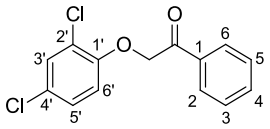
107

R_f: 0.92 (dichloromethane/methanol, 7:3)
m.p.: >180 ºC dec
IR (ATR): 3440 (OH, NH); 1636 (C=O)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.11 (s, 3H, CH_3); 4.38 (s, 2H, CH_2); 6.32 (dd, *J* = 8.7, 2.2, 1H, H_6); 6.46 (d, *J* = 2.2, 1H, H_2); 6.88-6.91 (m, 2H, $H_{2''}$, $H_{6''}$); 6.99 (t, *J* = 7.4, 1H, $H_{4''}$); 7.26-7.29 (m, 2H, $H_{3''}$, $H_{5''}$); 7.56 (d, *J* = 8.8, 1H, H_5)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 14.4 (CH_3); 68.2 (CH_2); 103.3 (C_2); 107.1 (C_6); 110.4 (C_4); 115.9 ($C_{2''}$, $C_{6''}$); 123.2 ($C_{4''}$); 128.7 (C_5); 130.8 ($C_{3''}$, $C_{5''}$); 133.5 ($C_{5'}$); 158.7 (C_3); 159.6 ($C_{1''}$); 160.5 (C_1); 177.1 (C=O); $C_{3'}$ and $C_{4'}$ not observed
HRMS (ESI, *m/z*): calculated for $C_{18}H_{15}N_2O_5$ $[M-H]^-$: 339.0986, found: 339.0975
HPLC (method C, t_R, min): 21.40

4.3.6. Synthesis of final compound **120**

2-(2,4-Dichlorophenoxy)-1-phenylethanone, 121

Following the general procedure 4.3.1.6., phenylethanone **121** was obtained from 2-bromoacetophenone (366 mg, 1.84 mmol) in 70% yield. Chromatography: hexane/ethyl acetate, 9:1 to hexane/ethyl acetate, 8:2.

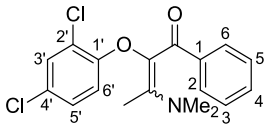


121

R_f: 0.50 (hexane/ethyl acetate, 5:1)
m.p.: 72-74 ºC
IR (ATR): 1700 (C=O); 1220 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.35 (s, 2H, CH_2); 6.78 (d, *J* = 8.8, 1H, $H_{6'}$); 7.14 (dd, *J* = 8.8, 2.5, 1H, $H_{5'}$); 7.39 (d, *J* = 2.5, 1H, $H_{3'}$); 7.49-7.54 (m, 2H, H_3, H_5); 7.61-7.66 (m, 1H, H_4); 7.99-8.01 (m, 2H, H_2, H_6)
MS (ESI, *m/z*): 281.0, 283.0, 285.1 $[M+H]^+$
HPLC (method A, t_R, min): 17.26

2-(2,4-Dichlorophenoxy)-3-(dimethylamino)-1-phenylbut-2-en-1-one, 122

Following the general procedure 4.3.1.7., enaminone **122** was obtained from phenylethanone **121** (90 mg, 0.32 mmol) in 55% yield. Chromatography: hexane/ethyl acetate, 1:1 to ethyl acetate.

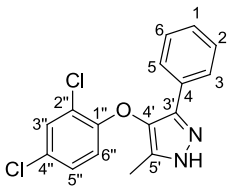


122

R_f: 0.35 (ethyl acetate)
IR (ATR): 1741 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ Mixture of isomers A:B (1:0.7): 2.06 (s, 3H, $CH_{3\ A}$); 2.45 (s, 3H, $CH_{3\ B}$); 3.01 (s, 6H, $N(CH_3)_{2\ B}$); 3.06 (s, 6H, $N(CH_3)_{2\ A}$); 6.77 (d, *J* = 8.9, 1H, $H_{6'\ B}$); 6.80 (d, *J* = 8.9, 1H, $H_{6'\ A}$); 6.99 (dd, *J* = 8.9, 2.4, 1H, $H_{5'\ A}$); 7.00 (dd, *J* = 8.9, 2.4, 1H, $H_{5'\ B}$); 7.16 (d, *J* = 2.4, 1H, $H_{3'\ B}$); 7.17 (d, *J* = 2.4, 1H, $H_{3'\ A}$); 7.21-7.30 (m, 3H, H_3, H_4, H_5); 7.63-7.71 (m, 2H, H_2, H_6)

4-(2,4-Dichlorophenoxy)-5-methyl-3-phenyl-1*H*-pyrazole, 120

Following the general procedure 4.3.1.5., pyrazole **120** was obtained from enaminone **122** (54 mg, 0.15 mmol) in 48% yield. Chromatography: toluene/acetone, 6:1 to toluene/acetone, 1:1.



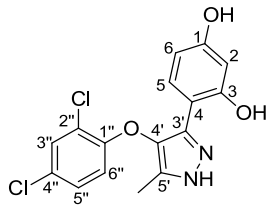
120

R_f: 0.41 (toluene/acetone, 1:1)
IR (ATR): 3280 (NH)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.14 (s, 3H, CH_3); 6.61 (d, *J* = 8.9, 1H, $H_{6''}$); 7.00 (dd, *J* = 8.9, 2.5, 1H, $H_{5''}$); 7.27-7.30 (m, 1H, H_4); 7.33-7.36 (m, 2H, H_3, H_5); 7.42 (d, *J* = 2.5, 1H, $H_{3''}$); 7.69-7.71 (m, 2H, H_2, H_6)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 9.7 (CH_3); 115.6 ($C_{6''}$); 123.3 ($C_{2''}$/$C_{4''}$); 125.9 (C_2, C_6); 127.4 ($C_{2''}$/$C_{4''}$); 127.9 ($C_{5''}$); 128.6 (C_4); 129.0 (C_3, C_5); 129.7 (C_1); 130.4 ($C_{3''}$); 133.5, 136.8 ($C_{4'}$, $C_{5'}$); 138.7 ($C_{3'}$); 152.7 ($C_{1''}$)
HRMS (ESI, *m/z*): calculated for $C_{16}H_{11}Cl_2N_2O$ $[M-H]^-$: 317.0254, found: 317.0266
HPLC (method A, t_R, min): 14.24

4.3.7. Synthesis of final compounds **123**, **124**

4-[4-(2,4-Dichlorophenoxy)-5-methyl-1*H*-pyrazol-3-yl]benzene-1,3-diol, 123

Following the general procedure 4.3.1.5., pyrazole **123** was obtained from **99** (82 mg, 0.24 mmol) in 65% yield. Chromatography: dichloromethane to dichloromethane/methanol, 8:2.



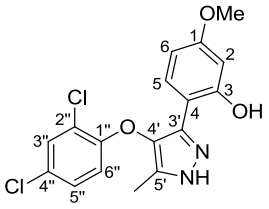
123

R_f: 0.23 (hexane/ethyl acetate, 6:4)
m.p.: 206-208 ºC
IR (ATR): 3293 (OH, NH)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 2.12 (s, 3H, CH_3); 6.21 (dd, *J* = 8.5, 2.1, 1H, H_6); 6.34 (d, *J* = 2.0, 1H, H_2); 6.66 (d, *J* = 8.9, 1H, $H_{6''}$); 7.11 (dd, *J* = 8.9, 2.4, 1H, $H_{5''}$); 7.40 (d, *J* = 8.6, 1H, H_5); 7.50 (d, *J* = 2.4, 1H, $H_{3''}$)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 8.6 (CH_3); 103.9 (C_2); 108.0 (C_6); 108.9 (C_4); 116.8 ($C_{6''}$); 124.1 ($C_{4''}$); 128.3 ($C_{2''}$); 128.6 (C_5); 129.1 ($C_{5''}$); 130.9 (C_{Ar}); 131.1 ($C_{3''}$); 133.0, 153.9 ($2C_{Ar}$); 158.1 ($C_{1''}$); 159.55, 159.63 (C_1, C_3)
HRMS (ESI, *m/z*): calculated for $C_{16}H_{13}Cl_2N_2O_3$ $([M+H]^+)$: 351.0298, found: 351.0323
HPLC (method C, t_R, min): 29.29

2-[4-(2,4-Dichlorophenoxy)-5-methyl-1*H*-pyrazol-3-yl]-5-methoxyphenol, 124

To a solution of pyrazole **123** (25 mg, 0.07 mmol, 1 equiv) in anhydrous acetone (0.8 mL) under an argon atmosphere, K_2CO_3 (21 mg, 0.15 mmol, 2.1 equiv) and iodomethane (18 mg, 0.13 mmol, 1.8 equiv) were added and the reaction was stirred at 65ºC for 6 h. After cooling to room temperature, the mixture was concentrated and

the residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (hexane to hexane/ethyl acetate, 7:3) to yield pure compound **124** in 19% yield.



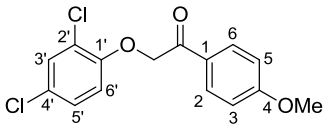
124

R_f: 0.50 (hexane/ethyl acetate, 6:4)
m.p.: 220-222 ºC
IR (ATR): 3352 (OH, NH)
^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.15 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3); 6.18 (dd, J = 8.6, 2.5, 1H, H_4); 6.31 (d, J = 2.4, 1H, H_6); 6.68 (d, J = 8.9, 1H, $H_{6''}$); 7.14 (dd, J = 8.9, 2.5, 1H, $H_{5''}$); 7.37 (d, J = 8.6, 1H, H_3); 7.53 (d, J = 2.5, 1H, $H_{3''}$)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 8.1 (CH_3); 37.1 (OCH_3); 104.0 (C_6); 107.9 (C_4); 109.1 (C_2); 116.8 ($C_{6''}$); 124.0 ($C_{4''}$); 128.3 (C_3); 128.4 ($C_{2''}$); 129.1 ($C_{5''}$); 131.2 ($C_{3''}$); 132.8, 133.2, 141.6 ($C_{3'}$, $C_{4'}$, $C_{5'}$); 153.9 ($C_{1''}$); 158.4, 159.5 (C_5, C_1)
HRMS (ESI, *m/z*): calculated for $C_{17}H_{15}Cl_2N_2O_3$ ([M+H]$^+$): 365.0454, found: 365.0485
HPLC (method C, t_R, min): 21.90

4.3.8. Synthesis of final compound **125**

2-(2,4-Dichlorophenoxy)-1-(4-methoxyphenyl)ethanone, 126

Following the general procedure 4.3.1.6., arylethanone **126** was obtained from 2-bromo-1-(4-methoxyphenyl)ethanone (1,69 g, 7.36 mmol) in 80% yield. Chromatography: hexane/ethyl acetate, 9:1 to hexane/ethyl acetate, 1:1.



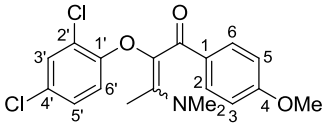
126

R_f: 0.40 (hexane/ethyl acetate, 5:1)
m.p.: 110-112 ºC
IR (ATR): 1691 (C=O); 1231 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 3H, CH_3); 5.27 (s, 2H, CH_2); 6.77 (d, J = 8.8, 1H, $H_{6'}$); 6.93-6.98 (m, 2H, H_3, H_5); 7.12 (dd, J = 8.8, 2.5, 1H, $H_{5'}$); 7.37 (d, J = 2.5, 1H, $H_{3'}$); 7.97-8.02 (m, 2H, H_2, H_6)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 55.7 (CH_3); 72.0 (CH_2); 114.2 (C_3, C_5); 115.0 ($C_{6'}$); 124.2, 126.9, 127.4 (C_1, $C_{2'}$, $C_{4'}$); 127.7 ($C_{5'}$); 130.4 ($C_{3'}$); 130.8 (C_2, C_6); 152.8, 164.4 (C_4, $C_{1'}$); 192.3 (C=O)
MS (ESI, *m/z*): 311.1, 313.0, 315.1 $[M+H]^+$
HPLC (method A, t_R, min): 17.81

2-(2,4-Dichlorophenoxy)-3-(dimethylamino)-1-(4-methoxyphenyl)but-2-en-1-one, 127

Following the general procedure 4.3.1.7., enaminone **127** was obtained from arylethanone **126** (263 mg, 0.85 mmol) in 52% yield. Chromatography: hexane/ethyl acetate, 1:1 to ethyl acetate.

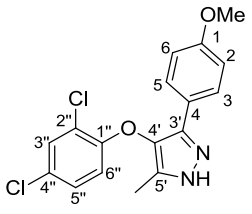


127

R_f: 0.30 (ethyl acetate)
IR (ATR): 1741 (C=O)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ Mixture of isomers A:B (1:0.8): 2.04 (s, 3H, $CH_{3\ A}$); 2.40 (s, 3H, $CH_{3\ B}$); 2.99 (s, 6H, $N(CH_3)_{2\ B}$); 3.02 (s, 6H, $N(CH_3)_{2\ A}$); 3.78 (s, 3H, OCH_3); 6.74-6.82 (m, 3H, H_3, H_5, $H_{6'}$); 6.99 (dd, *J* = 8.9, 2.5, 1H, $H_{5'\ A}$); 7.00 (dd, *J* = 8.9, 2.5, 1H, $H_{5'\ B}$); 7.19 (d, *J* = 2.2, 1H, $H_{3'\ B}$); 7.20 (d, *J* = 2.2, 1H, $H_{3'\ A}$); 7.72-7.78 (m, 2H, H_2, H_6)

4-(2,4-Dichlorophenoxy)-5-methyl-3-(4-methoxyphenyl)-1*H*-pyrazole, 128

Following the general procedure 4.3.1.5., pyrazole **128** was obtained from enaminone **127** (154 mg, 0.41 mmol) in 44% yield. Chromatography: hexane/ethyl acetate, 4:1 to hexane/ethyl acetate, 1:1.



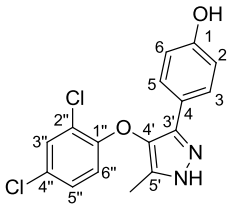
128

R_f: 0.16 (hexane/ethyl acetate, 1:1)
IR (ATR): 3133 (NH); 1251 (C-O-C)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.13 (s, 3H, CH_3); 3.79 (s, 3H, OCH_3); 6.60 (d, *J* = 8.9, 1H, $H_{6''}$); 6.85-6.87 (m, 2H, H_3, H_5); 7.00 (dd, *J* = 8.9, 2.5, 1H, $H_{5''}$); 7.42 (d, *J* = 2.5, 1H, $H_{3''}$); 7.60-7.63 (m, 2H, H_2, H_6)

^{13}C-NMR ($CDCl_3$, 125 MHz): δ 9.7 (CH_3); 55.4 (OCH_3); 114.5 (C_3, C_5); 115.6 ($C_{6''}$); 122.1 (C_1); 123.3 ($C_{2''}$/$C_{4''}$); 127.26 (C_2, C_6); 127.32 ($C_{2''}$/$C_{4''}$); 127.9 ($C_{5''}$); 130.4 ($C_{3''}$); 132.9, 137.0 ($C_{4'}$, $C_{5'}$); 138.3 ($C_{3'}$); 152.7 ($C_{1''}$); 159.9 (C_4)
MS (ESI, *m/z*): 349.1, 351.1, 353.0 $[M+H]^+$
HPLC (method A, t_R, min): 20.54

4-(2,4-Dichlorophenoxy)-3-(4-hydroxyphenyl)-5-methyl-1*H*-pyrazole, 129

To a solution of pyrazole **128** (49 mg, 0.14 mmol, 1 equiv) in anhydrous dichloromethane (3.0 mL) at -78°C under an argon atmosphere, BBr_3 (105 mg, 0.42 mmol, 3.0 equiv) was added and the mixture was stirred at -78°C for 1 h. After this time the reaction was warmed up to room temperature and stirred at this temperarure for 20 h. Then, the mixture was washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (hexane/ethyl acetate, 1:1 to 3:7) to yield pure compound **129** in 70% yield.



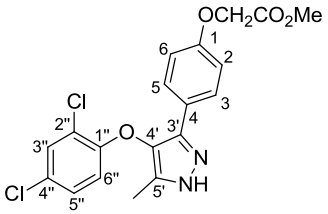
129

R_f: 0.54 (hexane/ethyl acetate, 1:3)
IR (ATR): 3267 (OH, NH); 1251 (C-O-C)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.14 (s, 3H, CH_3); 6.61 (d, *J* = 8.9, 1H, $H_{6''}$); 6.80 (d, *J* = 8.7, 2H, H_2, H_6); 7.02 (dd, *J* = 8.8, 2.5, 1H, $H_{5''}$); 7.42 (d, *J* = 2.4, 1H, $H_{3''}$); 7.54 (d, *J* = 8.6, 2H, H_3, H_5)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 9.7 (CH_3); 115.6 ($C_{6''}$); 116.0 (C_2, C_6); 122.2 (C_4); 123.3, 127.4 ($C_{2''}$, $C_{4''}$); 127.5 (C_3, C_5); 127.9 ($C_{5''}$); 130.4 ($C_{3''}$); 133.0, 136.9 ($C_{4'}$, $C_{5'}$); 138.6 ($C_{3'}$); 152.7 ($C_{1''}$); 156.1 (C_1)
MS (ESI, *m/z*): 333.0.1, 335.0, 336.9 $[M-H]^-$
HPLC (method A, t_R, min): 17.81

4-(2,4-Dichlorophenoxy)-3-(4-(methoxycarbonyl)methoxyphenyl)-5-methyl-1*H*-pyrazole, 130

To a solution of pyrazole **129** (31 mg, 0.09 mmol, 1 equiv) in anhydrous DMF (2.0 mL) under an argon atmosphere, K_2CO_3 (26 mg, 0.19 mmol, 2 equiv) and methyl 2-bromoacetate (0.009 mL, 0.09 mmol, 1 equiv) were added at -20°C. After 4 h at this temperature, the reaction was stirred overnight at room temperature. Then, another portion of methyl 2-bromoacetate (0.002 mL, 0.02 mmol, 0.2 equiv) was added and the mixture was stirred for 4 h. The reaction mixture was diluted with ethyl acetate,

washed with water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (toluene/acetone, 10:1 to toluene/acetone, 3:1) to yield pure compound **130** in 32% yield.

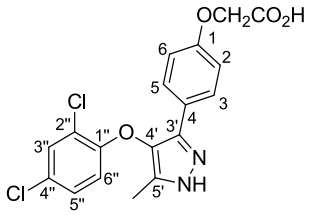


130

R_f: 0.49 (toluene/acetone, 1:1)
IR (ATR): 3133 (NH); 1761 (C=O)
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.14 (s, 3H, CH_3); 3.79 (s, 3H, OCH_3); 4.62 (s, 2H, CH_2); 6.61 (d, *J* = 8.9, 1H, $H_{6''}$); 6.88 (d, *J* = 9.0, 2H, H_2, H_6); 7.02 (dd, *J* = 8.9, 2.5, 1H, $H_{5''}$); 7.42 (d, *J* = 2.5, 1H, $H_{3''}$); 7.63 (d, *J* = 9.0, 2H, H_3, H_5)
^{13}C-NMR ($CDCl_3$, 125 MHz): δ 9.7 (CH_3); 52.5 (OCH_3); 65.4 (CH_2); 115.2 (C_2, C_6); 115.6 ($C_{6''}$); 123.3 ($C_{2''}$/$C_{4''}$); 123.5 (C_4); 127.35 (C_3, C_5); 127.40 ($C_{2''}$/$C_{4''}$); 127.9 ($C_{5''}$); 130.4 ($C_{3''}$); 133.1, 136.5 ($C_{4'}$, $C_{5'}$); 138.6 ($C_{3'}$); 152.7 ($C_{1''}$); 158.0 (C_1); 169.3 (C=O)
MS (ESI, *m/z*): 407.1, 409.1, 411.0 $[M+H]^+$
HPLC (method A, t_R, min): 12.86

4-(2,4-Dichlorophenoxy)-3-(4-carboxymethoxyphenyl)-5-methyl-1*H*-pyrazole, 125

A mixture of pyrazole **130** (10 mg, 0.02 mmol, 1 equiv) and 1M NaOH (0.03 mL, 0.02 mmol, 1.2 equiv) in 1,4-dioxane (1 mL) was stirred at 60°C for 4 h under an argon atmosphere. After cooling to room temperature, the mixture was neutralized with 1M HCl, and extracted with ethyl acetate (2x). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (ethyl acetate/methanol, 8:2 to ethyl acetate/methanol, 7:3) to yield pure compound **125** in 99% yield.



125

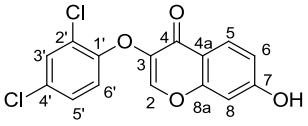
R_f: 0.13 (ethyl acetate/methanol, 7:3)
m.p.: 158-160 ºC
IR (ATR): 3321 (NH, OH)

^{1}H-NMR (methanol-d_4, 700 MHz): δ 2.11 (s, 3H, CH_3); 4.63 (s, 2H, CH_2); 6.66 (d, *J* = 8.9, 1H, $H_{6''}$); 6.91-6.94 (m, 2H, H_2, H_6); 7.12 (dd, *J* = 8.9, 2.5, 1H, $H_{5''}$); 7.50 (d, *J* = 2.5, 1H, $H_{3''}$); 7.61-7.63 (m, 2H, H_3, H_5)
^{13}C-NMR (methanol-d_4, 175 MHz): δ 9.2 (CH_3); 66.1 (CH_2); 115.9 (C_2, C_6); 116.7 ($C_{6''}$); 124.1 ($C_{2''}$/$C_{4''}$); 124.4 (C_4); 128.2 (C_3, C_5); 128.3 ($C_{2''}$/$C_{4''}$); 129.1 ($C_{5''}$); 131.1 ($C_{3''}$); 133.7, 137.7 ($C_{4'}$, $C_{5'}$); 139.2 ($C_{3'}$); 154.1 ($C_{1''}$); 159.5 (C_1); 172.9 (C=O)
HRMS (ESI, *m/z*): calculated for $C_{18}H_{13}Cl_2N_2O_4$ ([M-H]$^-$): 391.0258, found: 391.0247
HPLC (method A, t_R, min): 17.53

4.3.9. Synthesis of final compound **133**

3-(2,4-Dichlorophenoxy)-7-hydroxy-4*H*-chromen-4-one, 131

A mixture of 2,4-dihydroxyphenyl ketone **97** (40 mg, 0.13 mmol, 1 equiv) and $BF_3{\cdot}Et_2O$ (0.02 mL, 0.19 mmol, 1.5 equiv) in anhydrous DMF (1 mL), was stirred at room temperature for 1 h under an argon atmosphere. Then, the reaction was heated at 50°C, methanesulphonyl chloride (0.02 mL, 0.23 mmol, 1.8 equiv) was added and the mixture was stirred at 100°C for 90 min. After cooling to room temperature, the mixture was diluted with ethyl acetate and washed with a 1:1 mixture of water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (hexane/ethyl acetate, 8:2 to hexane/ethyl acetate, 4:6) to yield pure compound **131** in 97% yield.

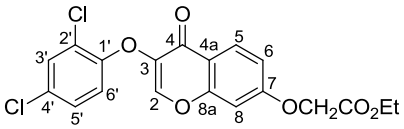


131

R_f: 0.60 (hexane/ethyl acetate, 1:2)
IR (ATR): 3091 (OH); 1629 (C=O); 1263 (C-O-C)
^{1}H-NMR (methanol-d_4, 300 MHz): δ 6.91 (d, *J* = 8.8, 1H, $H_{6'}$); 6.91 (d, *J* = 2.2, 1H, H_8); 6.97 (dd, *J* = 8.8, 2.2, 1H, H_6); 7.19 (dd, *J* = 8.9, 2.5, 1H, $H_{5'}$); 7.50 (d, *J* = 2.5, 1H, $H_{3'}$); 8.01 (d, *J* = 8.8, 1H, H_5); 8.36 (s, 1H, H_2)
^{13}C-NMR (methanol-d_4, 75 MHz): δ 103.6 (C_8); 116.8 (C_6); 118.0 ($C_{6'}$); 118.3 (C_{4a}); 124.8 ($C_{2'}$/$C_{4'}$); 128.2 (C_5); 128.9 ($C_{5'}$); 129.0 ($C_{2'}$/$C_{4'}$); 131.1 ($C_{3'}$); 140.8 (C_3); 150.8 (C_2); 153.6 ($C_{1'}$); 159.8 (C_{8a}); 165.1 (C_7); 173.6 (C_4)
MS (ESI, *m/z*): 321.0, 323.0, 324.9 [M-H]$^-$
HPLC (method C, t_R, min): 28.80

Ethyl {[3-(2,4-dichlorophenoxy)-4-oxo-4*H*-chromen-7-yl]oxyacetate, 132

Following the general procedure 4.3.1.4., compound **132** was obtained from hydroxychromone **131** (35 mg, 0.11 mmol) and ethyl 2-bromoacetate (0.04 mL, 0.39 mmol) in 91% yield.

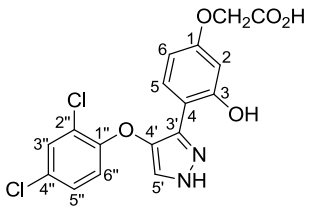


132

R_f: 0.50 (hexane/ethyl acetate, 2:1)
IR (ATR): 1752 (C=O); 1653 (C=O); 1196 (C-O-C)
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.32 (t, *J* = 7.1, 3H, CH_3); 4.30 (q, *J* = 7.1, 2H, CH_2CH_3); 4.73 (s, 2H, CH_2); 6.82 (d, *J* = 8.8, 1H, $H_{6'}$); 6.88 (d, *J* = 2.4, 1H, H_8); 7.05 (dd, *J* = 9.0, 2.4, 1H, H_6); 7.11 (dd, *J* = 8.8, 2.5, 1H, $H_{5'}$); 7.44 (d, *J* = 2.5, 1H, $H_{3'}$); 8.03 (s, 1H, H_2); 8.17 (d, *J* = 9.0, 1H, H_5)
^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.3 (CH_3); 62.0 (CH_2CH_3); 65.6 (CH_2), 101.7 (C_8); 115.0 (C_6); 117.4 ($C_{6'}$); 119.4 (C_{4a}); 124.4 ($C_{2'}$/$C_{4'}$); 127.8 ($C_{5'}$); 128.0 (C_5); 128.6 ($C_{2'}$/$C_{4'}$); 130.5 ($C_{3'}$); 140.6 (C_3); 147.9 (C_2); 151.8 ($C_{1'}$); 157.6 (C_{8a}); 162.5 (C_7); 167.8 (C=O), 171.6 (C_4)
MS (ESI, *m/z*): 408.9, 410.9, 412.9 $[M+H]^+$
HPLC (method A, t_R, min): 17.52

{4-[4-(2,4-Dichlorophenoxy)-1*H*-pyrazol-3-yl]-3-hydroxyphenoxy}acetic acid, 133

Following the general procedure 4.3.1.5., pyrazole **133** was obtained from chromone **132** (27 mg, 0.07 mmol) in 58% yield. Chromatography: ethyl acetate/methanol, 9:1 to ethyl acetate/methanol, 8:2.



133

R_f: 0.08 (ethyl acetate/methanol, 7:3)
IR (ATR): 3187 (OH, NH); 1627 (C=O)
^{1}H-NMR (methanol-d_4, 500 MHz): δ 4.42 (s, 2H, CH_2); 6.39 (dd, *J* = 8.8, 2.5, 1H, H_6); 6.49 (d, *J* = 2.5, 1H, H_2); 6.84 (d, *J* = 8.9, 1H, $H_{6''}$); 7.16 (dd, *J* = 8.9, 2.5, 1H, $H_{5''}$); 7.50 (d, *J* = 2.5, 1H, $H_{3''}$); 7.57 (d, *J* = 8.7, 1H, H_5); 7.61 (br s, 1H, $H_{5'}$)
^{13}C-NMR (methanol-d_4, 125 MHz): δ 67.8 (CH_2); 103.5 (C_2); 107.2 (C_6); 110.1 (C_4); 117.8 ($C_{6''}$); 124.6 ($C_{2''/4''}$); 128.7 (C_5); 128.9 ($C_{2''/4''}$); 129.1 ($C_{5''}$); 131.1 ($C_{3''}$); 136.6 ($C_{4'}$); 154.4 ($C_{1''}$); 157.9 (C_3); 160.8 (C_1); 176.4 (C=O); $C_{3'}$ and $C_{5'}$ not observed
HRMS (ESI, *m/z*): calculated for $C_{17}H_{11}Cl_2N_2O_5$ ($[M-H]^-$): 393.0050, found: 393.0017
HPLC (method A, t_R, min): 21.92

4.4. Biological assays

4.4.1. General reagents

Collagen, poly-D-lysine, poly-L-lysine and LPA were purchased from Aldrich. Ionomycin was purchased from Cayman. RH7777 hepatoma cells stably expressing the LPA_1 receptor and their corresponding non-transfected controls were kindly provided by Prof. Gabor Tigyi (University of Tennessee Health Science Center, Memphis, Tennessee, USA). B103 neuroblastoma cells stably expressing the LPA_2 or LPA_3 receptors were provided by Prof. Jerold Chun (The Scripps Research Institute, La Jolla, California, USA). Retrovirus expression vector (LZRS-EGFP) and Phoenix retrovirus producer cell lines were provided by Prof. Garry P. Nolan (Stanford University, California, USA).

4.4.2. Cell culture

All reagents were from Gibco. All cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, 1% sodium pyruvate, 100 U/mL penicillin, and 100 µg/mL streptomycin in a 5% CO_2 humidified atmosphere at 37ºC. For passage, cells were rinsed with phosphate buffered saline (PBS) and incubated with 0.125% trypsin, 0.02% EDTA solution for 2 min at 37ºC. Detached cells were resuspended in growth medium, counted if necessary, and splitted onto dishes containing fresh media.

4.4.3. Evaluation of receptor activation by Ca^{2+} mobilization assay

Changes in intracellular calcium levels were measured by using the fluorescent calcium sensitive dye Fluo-4NW (Invitrogen). RH7777 cells or B103 cells were plated on poly-D-lysine or collagen-coated, respectively, black-wall clear-bottom 96-well plates (Corning) at a density of 50000 cells/well and cultured overnight. The culture medium was then replaced with Fluo-4 NW dye loading solution containing 2.5 µM of probenecid and incubated for 30 min at 37ºC followed by an additional 30 min at room temperature. Fluorescence changes were registered in a FluoStar Optima instrument (BMG Labtech) at 525 nm using an excitation wavelength of 494 nm. Each well was monitored for 240 s. 20 µL of the test compound from a 6x stock solution in assay buffer were added after 120 s of starting the measurement. Ca^{2+} transient increase was quantified by calculating the difference between maximum and baseline values for each well. As positive controls, 10 µM LPA and 10 µM ionomycin were included in every experiment. At this concentration, LPA induced a response about 30-33% of the one shown by ionomycin, which is in agreement with previously described results.[87]

The data presented are from two to four independent experiments carried out in triplicate or quadruplicate. Dose-response curves were generated and EC_{50} and IC_{50}

values calculated by nonlinear regression analysis using PRISM software version 5 (GraphPad Software Inc, San Diego, CA, USA).

4.4.4. BSI experiments

BSI utilizes a red helium-neon (HeNe) laser (λ = 632.8 nm) to illuminate the microfluidic channel in a simple optical train. The laser is coupled to a collimating lens through a single-mode fiber, producing a 100 µm diameter beam and yielding probe volumes in the 300 picoliter range. When the laser beam intersects the fluid contained in the channel, a set of high-contrast interference fringes is produced, the spatial position of which depends on the RI of the fluid within the channel. The fringes were monitored in the direct backscatter region at relatively shallow angles (typically <7°) using a Garry 3000 linear array CCD camera (Ames Photonics). The camera was positioned so that the centroid of the interference pattern was located just above the lens on the end of the single-mode optical fiber to ensure that the alignment was along a central plane. Under these conditions the fringe pattern contains a dominant Fourier frequency; the phase of this dominant frequency is the BSI fringe shift signal, in radians.

BSI chips were isotropically etched in borosilicate glass to give a cross-section described by two quarter-circles of 40 µm radius connected by a 10 µm flat region, manufactured by Micronit. For BSI measurements, the chip was maintained in the instrument at 25°C using a feedback-controlled peltier system.

A cell pellet containing approximately 10^6 cells was re-suspended in 1 mL of ice-cold protease inhibitor cocktail in Dulbecco's PBS (Gibco) and sonicated during 1 min and 30 s at 4°C. This solution was then centrifuged for 10 min at 10000*g* and at 4°C, and the resulting supernatant was centrifuged for another 50 min at 10000*g* and at 4°C. Protein concentration was determined, and 50 µL aliquots (protein concentration = 0.16 mg/mL) were mixed with 50 µL of a solution that contained the appropriate ligand concentration (0 to 0.5 µM) in buffer (Dulbecco's PBS). 1 µL of each sample was introduced into the microfluidic channel for each measurement, of which 290 picoliters are interrogated by the laser. Five aliquots containing different concentrations of ligand were used to generate a complete binding curve and to determine the K_D value, using GraphPad Prism software.

4.4.5. Receptor internalization assay

B103 neuroblastoma cells overexpressing LPA_1 receptor were plated on poly-L-lysine-coated glass coverslips in 24-well plates ($5 \cdot 10^4$ cells per well) and serum-starved overnight. For the experiment, media was replaced with 1 mL fresh serum-free DMEM, supplemented with 0.1% FAF BSA, 1 µM LPA, or compound. After 15 min, cells were fixed with 4% paraformaldehyde and permeabilized with 0.025% Triton X-100/PBS for 15 min. F-actin was detected with rhodamine-phalloidin (Sigma)

in PBS and nuclei with DAPI (Sigma). For receptor internalization, cells were analysed using confocal fluorescence microscopy (Carl Zeiss).

4.4.6. Receptor desensitization assay

4.4.6.1. Primary culture of sensory neurons

DRG were harvested from neonatal Wistar rats (3-5 days old). Ganglia were digested with 0.25% (w/v) collagenase (type IA) in DMEM-glutamax (Invitrogen) with 1% P/S (5000 U/mL, Invitrogen) for 1 h (37ºC, 5% CO_2). After digestion, rat DRG was mechanically dissociated using a glass Pasteur pipette. Single cell suspension was passed through a 100 µm cell strainer, and washed with DMEM glutamax plus 10% FBS (Invitrogen) and 1% P/S. Cells were seeded at the 30 µL of medium containing cells on microelectrode array chambers previously coated with poly-L-lysine (8.33 µg/mL) and laminin (5 µg/mL). After 2 h, medium was replaced with DMEM glutamax, 10% FBS and 1% P/S, supplemented with 50 ng/mL mouse nerve growth factor 2.5s (Promega), and 1.25 µg/mL cytosine arabinoside when required (37ºC, 5% CO_2). All experiments were made 48 h after cell seeding.

4.4.6.2. Microelectrode Array (MEA)

Extracellular recordings were made using multiple electrode planar arrays of 60-electrode thin MEA chips, with 30 µm diameter electrodes and 200 µm inter-electrode spacing with an integrated reference electrode (Multichannel Systems GmbH). The electrical activity of primary sensory neuron was recorded by the MEA1060 System (Multi Channel Systems GmbH), and MC_Rack software version 4.3.0 at a sampling rate of 25 kHz. LPA and **(*S*)-3a** were perfused at 10 µM for 15 s to identify evoked neuronal spikes on cultured rat DRG neurons, using continuous perfusion system (2 mL/min flux). Capsaicin (500 nM) was applied at the end of the protocol to measure TRPV1 sensitivity of the neurons which were exposed to LPA or **(*S*)-3a**. Data were analyzed using MC_RACK spike sorter and Neuroexplorer Software (Nex Technologies). An evoked spike was defined when the amplitude of the neuronal electrical activity overcame a threshold set at -20 µV. The recorded signals were then processed to extract mean spike frequency.

4.4.6.3. Calcium microfluorography

DRG on coverslips loaded with 5 µM fluo-4-acetoxymethyl ester (Fluo-4AM) plus 0.02% pluronic acid (Molecular Probes, Invitrogen) in HBSS extracellular solution (140 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 D-glucose), and 10 HEPES, pH 7.4 for 1 h (37°C, 5% CO_2) were mounted in a RC-25 chamber (Harvard Apparatus), and continuously perfused with test solutions at room temperature. Fluorescence from individual neurons monitored through a 10x air objective (Aixiovert 200 inverted microscope, Carl Zeiss) with an ORCA-ER CCD camera (Hamamatsu Photonics). Pre-programed protocols applied via computer-controlled pinch valves (Bioscience

Tools, 5 mL/min flux). Fluo-4AM excited at 500 nm and emitted fluorescence filtered at 535 nm (Lambda-10-2-filter wheel, Sutter Instruments). Images processed with AquaCosmos package software (Hamamatsu Photonics). LPA and **(*S*)-3a** were perfused at 10 µM for 10 s. TRPV1 channel activity was evoked with 10 s-application of Caps at 1 µM. 10 s pulse of 40 mM KCl was applied to distinguish neuronal viability. Ionomycin was used at 5 µM. Intracellular Ca^{2+} increase calculated as fluorescence difference between baseline and the peak reached. An evoked response defined when intensity overcame 20% of baseline.

4.4.7. PAMPA assay

The assessment of the membrane permeability of ligand **103** and reference compounds propranolol and metoprolol was performed in a commercially available 96-well Corning Gentest pre-coated PAMPA plate system (Cultek S.L.U., Spain). Prior to use, the pre-coated PAMPA plate system was warmed to room temperature for 30 min and 300 µL of 200 µM solution of tested compound in 2% DMSO in PBS (pH 7.4) were added into wells in the receiver (donor) plate. Then 200 µL of PBS were added into wells in the filter (acceptor) plate. The filter plate was placed on the receiver plate by slowly lowering the pre-coated PAMPA plate until it sits on the receiver plate. The assembly was incubated at room temperature for 5 h, and then buffer samples were collected carefully from each plate. The final concentrations of compound in both donor and acceptor wells were analyzed by HPLC-MS and quantification was estimated by using the peak area integration normalized with an internal standard. Permeability value of the compounds was calculated using the following formula: $P\ (cm/s) = \{-\ln[1-C_A(t)/C_{eq}]\}/[A*(1/V_D+1/V_A)*t]$, where A = filter area (0.3 cm_2), V_D = donor well volume (0.3 mL), V_A = acceptor well volume (0.2 mL), t = incubation time (s), $C_A(t)$ = compound concentration (µM) in acceptor well at time t, $C_D(t)$ = compound concentration (µM) in donor well at time t, and $C_{eq} = [C_D(t)*V_D+C_A(t)*V_A]/(V_D+V_A)$. Assays were performed in duplicate and the compound was tested in two different plates on different days.

SUMMARY

5. SUMMARY

5.1. Introduction and Objectives

Lysophospholipids (LPs) are cell membrane lipid derivatives that also act as extracellular signals and play important roles in humans and other mammals. Analysis of LPs in human body fluids from subjects with different pathophysiological conditions reveal not only the relevance of LPs and their receptors in human diseases, but also their potential application as biomarkers and/or therapeutic targets.[1,2] Among them, lysophosphatidic acid (LPA) stands out as a molecule that elicits a plethora of biological effects by binding to specific G protein-coupled receptors: $LPA_{1–6}$ receptors.[3-5]

LPA regulates a wide variety of biological activities, notably within the developing and adult nervous system.[6,7] Considering the pleiotropic effects of LPA on many nervous system cell types, together with data showing localized up-regulation of LPA receptors after injury in mice and humans, it is likely that LPA regulates essential aspects of the cellular reorganization after neural trauma.[8] Among all neuropathologies where LPA plays an important role, neurophatic pain (NP)[9-13] and spinal cord injury (SCI)[14-16] are high incidence and seriously disabling conditions which currently lack specific pharmacological therapies. In this context, LPA_1 and LPA_2 receptor subtypes have been functionally linked to many neural processes associated to these pathologies,[17,18] but the lack of potent and selective agonists and antagonists has impaired the delineation of their specific role. Hence, we have focused our efforts on the development of such agents to clarify the biological role of LPA_1 and LPA_2 receptors in NP and SCI.

This overall objective involves the following steps:

1. Design and synthesis of new ligands with agonist or antagonist activity at LPA_1 and LPA_2 receptors.
2. Determination of the activity and selectivity at LPA receptors.
3. Biological validation of the selected compounds.

5.2. Results and Discussion

5.2.1. Development of new agonists for the LPA_1 receptor

In the case of NP, we proposed a stategy based on targeting LPA_1 receptor with a selective agonist, which, after activation, should promote receptor desensitization and internalization, and hence, could produce long-lasting antinociceptive effects.[19-21]

In order to find new molecular entities with agonist activity at LPA_1, we used the structure of the endogenous ligand, LPA, as starting point. Initially, two series of compounds were designed: series **I**, which comprised changes in the acid group attached to the glycerol backbone of LPA, and series **II**, which included modifications in the hydrophobic chain (Figure 1).

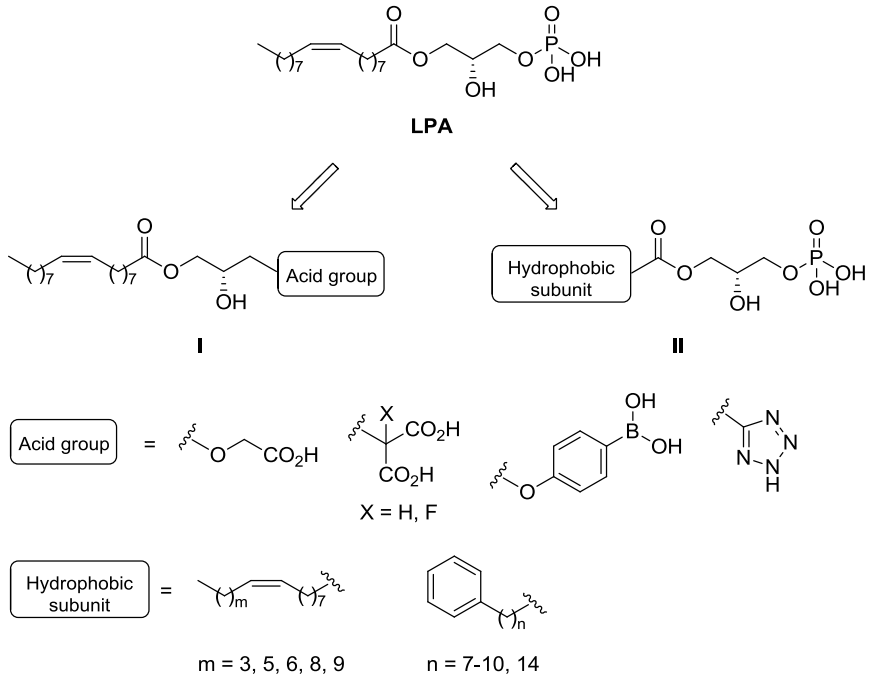
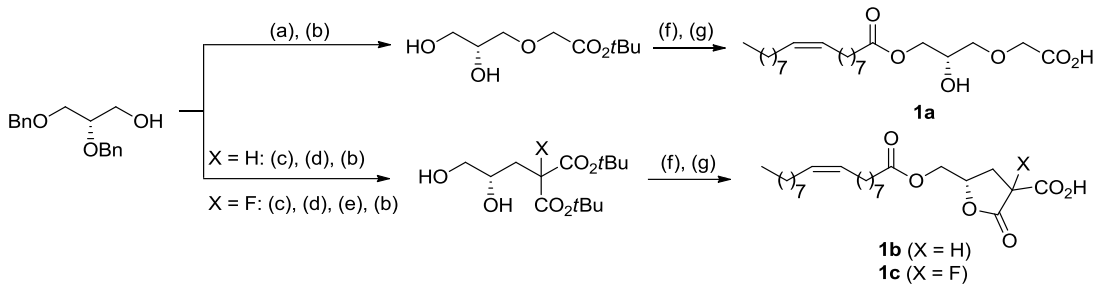
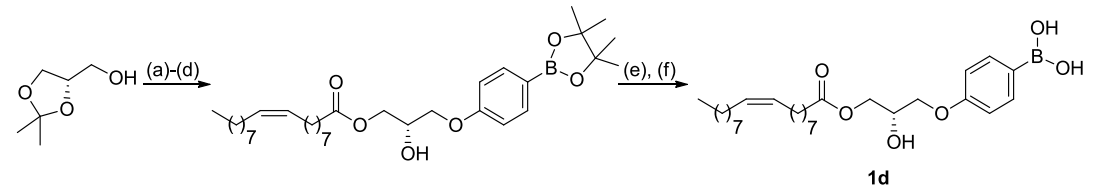


Figure 1. Design of new LPA_1 ligands.

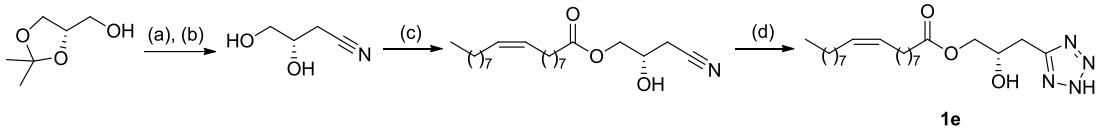
Regarding series **I**, it has been shown that changes in the polar head of LPA are poorly tolerated, so phosphate mimetics, specifically carboxylic and boronic acids, and tetrazole were chosen as acidic replacements.[22] Compounds belonging to series **I** were synthesized according to Schemes 1-3. It must be noted that the reaction conditions employed for the obtention of these derivatives led to the formation of lactones **1b** and **1c** instead of the expected malonic acid derivatives (Scheme 1).



Scheme 1. Reagents and conditions: (a) *tert*-Butyl bromoacetate, NaH, TBAI, THF, 0ºC to 50ºC, 16 h, 22%; (b) H_2, 10% Pd(C), EtOH, 60ºC, 89-95%; (c) mesyl chloride, Et_3N, DCM, 0ºC to rt, 1 h, 80%; (d) di-*tert*-butyl malonate, NaH, NaI, DMF:THF, 0ºC to 80ºC, 17 h, 76%; (e) Selectfluor®, NaH, THF:DMF, 48 h, 0ºC to rt, 99%; (f) oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 36-99%; (g) TFA, DCM, rt, 17-18 h, 52-99%.



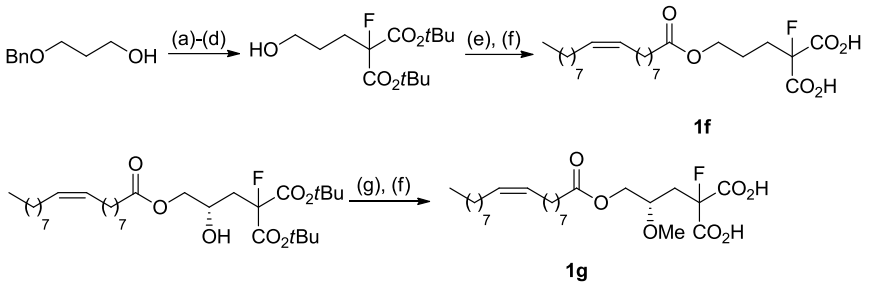
Scheme 2. Reagents and conditions: (a) Tosyl chloride, pyridine, DCM, 0ºC to rt, 16 h, 86%; (b) 4-hydroxyphenylboronic acid pinacol ester, Cs_2CO_3, DMF, 90ºC, 16 h, 84%; (c) PS-*p*TsOH, CH_3OH, rt, 18 h, 88%; (d) oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 40%; (e) KHF_2, CH_3OH:H_2O, rt, 30 min; 99%; (f) TMSCl, CH_3CN:H_2O, rt, 1 h, 60%.



Scheme 3. Reagents and conditions: (a) i) Pyridine, triflic anhydride, DCM, -20ºC, 30 min; ii) KCN, CH_3CN:H_2O, rt, 12 h, 99%; (b) TFA, CH_3OH, rt, 1.5 h, 61%; (c) oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 12 h, 88%; (d) NaN_3, NH_4Cl, DMF, MW, 160ºC, 45 min, 5%.

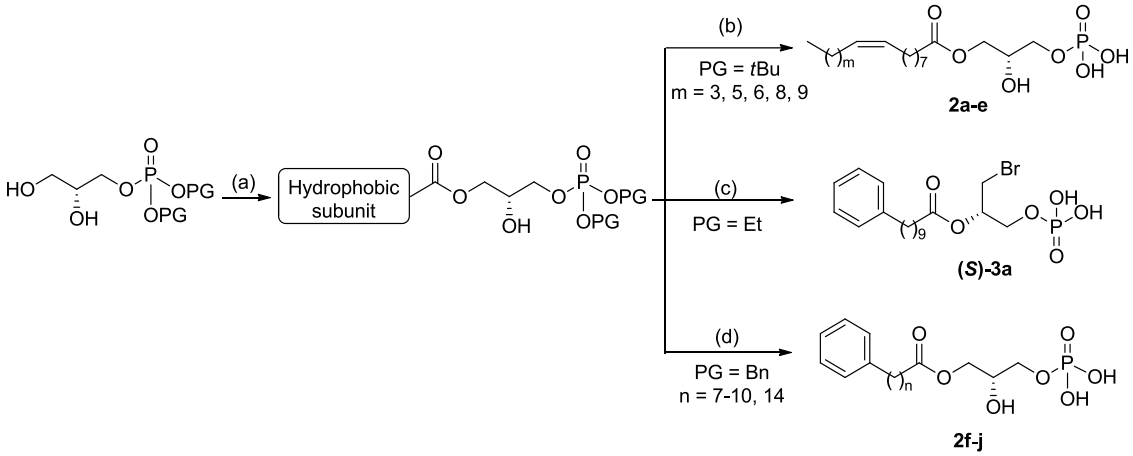
In order to determine the agonist capacity of the synthesized compounds, calcium mobilization assays in RH7777 cells stably transfected with LPA_1 were carried out. Activation of LPA_1 receptor induces a transient increase in the intracellular calcium levels which can be detected by fluorescence.

From this initial set of compounds only compound **1c** activated the receptor, with a maximal receptor activation (E_{max}) at 10 µM of 33% and an half maximal effective concentration (EC_{50}) value of 1.7 µM. Accordingly, it was necessary to confirm if the non-cyclic α-fluoromalonic derivative originally proposed, with two free carboxylic acids, would exhibit better activity at LPA_1 receptor. Therefore, compounds **1f** and **1g** were synthesized (Scheme 4). Only compound **1g** showed agonist activity with an EC_{50} value of 6 µM.



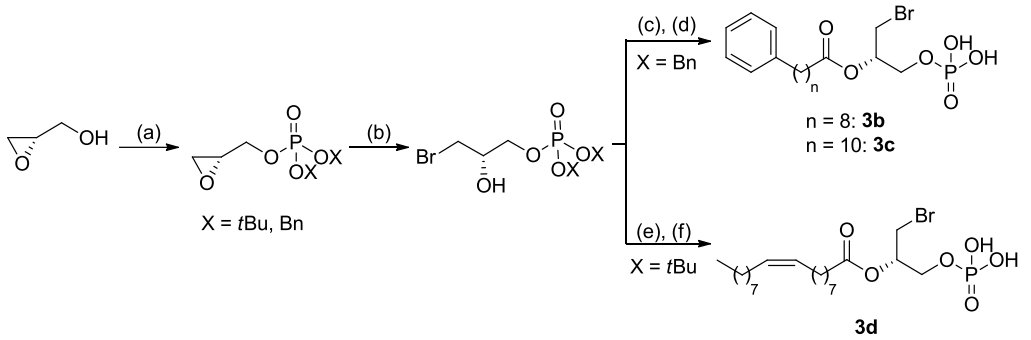
Scheme 4. Reagents and conditions: (a) Mesyl chloride, Et_3N, DCM, 0ºC to rt, 1 h, 99%; (b) di-*tert* butyl malonate, NaH, NaI, DMF:THF, 0ºC to 80ºC, 17 h, 66%; (c) Selectfluor®, NaH, THF:DMF, 48 h, 0ºC to rt, 99%; (d) H_2, 10% Pd(C), EtOH, 60ºC, 95%; (e) oleoyl chloride, 2,4,6-collidine, DCM, -78ºC to rt, 24 h, 70%; (f) TFA, DCM, rt, 16-17 h, 90-95%; (g) trimethylsilyldiazomethane, HBF_4, DCM, 0ºC, 90 min, 38%.

Regarding series **II**, a comprehensive study of the influence of the hydrophobic moiety was carried out, including modifications on the overall length of the fatty acid chain as well as the incorporation of an aromatic ring. Compounds belonging to series **II** were synthesized according to Scheme 5.



Scheme 5. Reagents and conditions: (a) Carboxylic acid, DCC, DMAP, DCM, -20ºC to rt, 16 h, 12-58%; (b) TFA, DCM, rt, 4-5 h, 90-99%; (c) i) TMSBr, DCM, rt, 4 h; ii) MeOH/H_2O, rt, 1h, 90%; (d) H_2, 10% Pd(C), EtOH, rt, 80-99%.

Compounds **2a**-**j** and the unexpectedly obtained **(*S*)-3a** were tested for their agonist activity at the LPA_1 receptor. The most remarkable conclusion that can be drawn from these data is the great influence on activity exerted by the length of the hydrophobic chain. From all the tested compounds, derivatives **2b** (m = 5), **2h** (n = 9) and **(*S*)-3a**, with E_{max} > 100% and EC_{50} values of 0.45, 0.5 and 0.24 µM respectively, stand out. Considering the excellent activity of compound **(*S*)-3a**, additional structural exploration was extended around this scaffold (Scheme 6). The obtained results showed that only **3d** was able to activate the receptor (E_{max} = 39%, EC_{50} = 3.2 µM), although it did not reach the values of compound **(*S*)-3a**.



Scheme 6. Reagents and conditions: (a) i) $(i\text{Pr})_2\text{NP(OX)}_2$, 1*H*-tetrazole, DCM, rt, 2 h; ii) *m*CPBA, -30ºC, 90 min, 52-76%; (b) TBABr, TFA, $CHCl_3$, rt, 10 min, 89-95%; (c) $\text{Ph(CH}_2)_n\text{COOH}$ (n = 8, 10), DCC, DMAP, DCM, rt, 16-18 h, 55-96%; (d) H_2, 10% Pd(C), EtOH, rt, 72-86%; (e) oleoyl chloride, pyridine, rt, 18 h, 15%; (f) TFA, DCM, rt, 5 h, 81%.

Selectivity of the identified LPA_1 agonists over LPA_2 and LPA_3 receptors

Taking into account the high sequence homology among the LPA_{1-3} receptors, we determined the selectivity of the best agonists of each series [**1c**, **1g**, **2b**, **2h** and **(*S*)-3a**]. Lack of activity at LPA_3 receptor is especially important, given that the activation of this receptor is implicated in the production of high LPA levels after peripheral nerve damage, responsible for initiation and maintenance of NP. Among the identified agonists, compounds **1c**, **1g**, **2b** and **(*S*)-3a** resulted selective for LPA_1 versus LPA_2 and LPA_3 receptors (Figure 2).

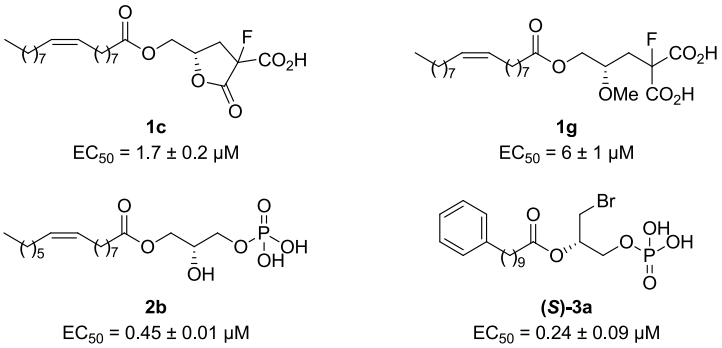


Figure 2. LPA_1 receptor agonists with selectivity versus LPA_2 and LPA_3 receptors.

Combination of the acid and hydrophobic subunits

Next, we combined the best hydrophobic subunits and the acid moieties that seem to be able to mimic the phosphate group, and have yielded the most potent agonists [**1c**, **1g**, **2b** and **(*S*)-3a**, Figure 2].

Hence, the lactone, the malonic acid, and the bromo-phosphate moieties present in derivatives **1c**, **1g**, and **(*S*)-3a**, respectively, were selected as acid groups. Regarding the hydrophobic subunit, oleoyl (present in **1c** and **1g**), palmitoleoyl (present in **2b**) and 10- phenyldecanoyl chains [present in **(*S*)-3a**] were chosen. The combination of these fragments led to products **1c**, **1g**, **(*S*)-3a**, and **3d** (which were previously synthesized) and new derivatives **4a**-**e** (Figure 3) which were synthesized

and tested as agonists of the LPA_1 receptor. From this new series, only derivative **4c** presented activity for LPA_1 receptor (E_{max} = 43%, EC_{50} = 1.4 μM).

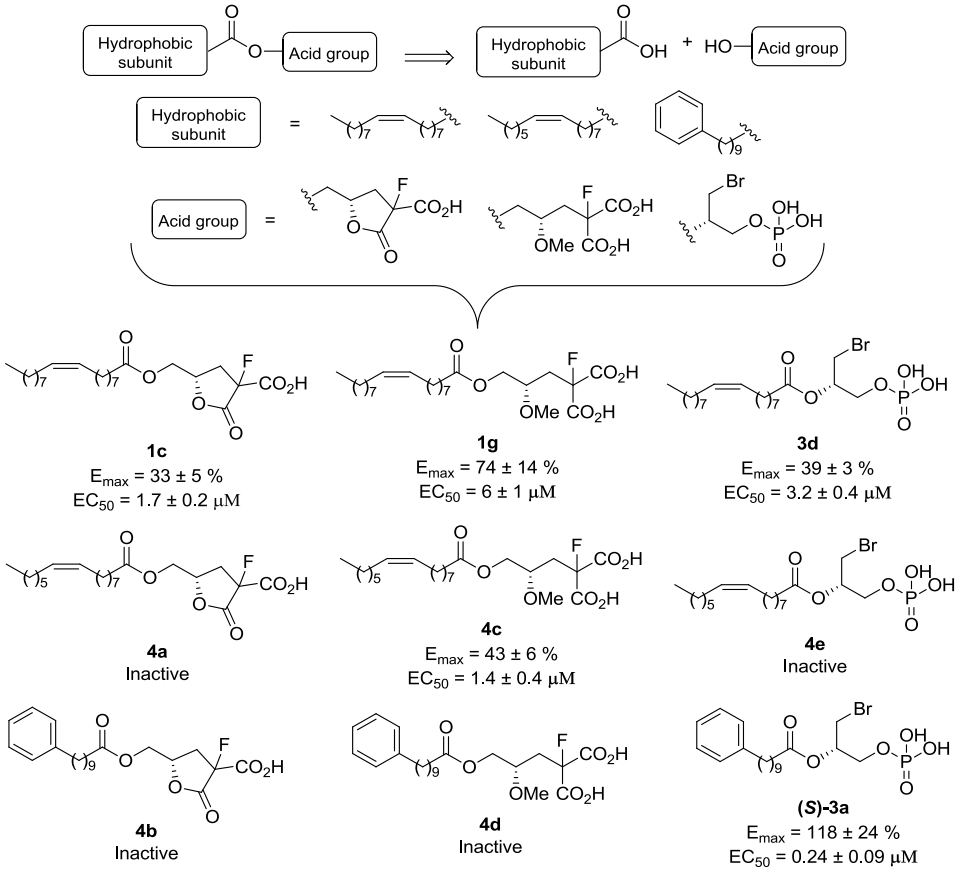
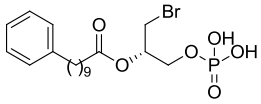
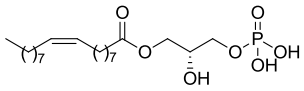


Figure 3. Combination of the optimal acid and hydrophobic subunits.

Among all the synthesized compounds, derivative **(*S*)-3a** stands out as the first agonist structurally different from the endogenous ligand LPA, with an excellent activity value and selectivity for LPA_1 versus LPA_2 and LPA_3 receptors (Table 1).

Table 1. Agonist activity of compound **(*S*)-3a** and endogenous ligand LPA at LPA_{1-3} receptors

Compound	EC_{50} (μM)[a] [E_{max} (%)][b]		
	LPA_1 receptor	**LPA_2 receptor**	**LPA_3 receptor**
 **(*S*)-3a**	0.24 ± 0.09 [118 ± 24]	N. E.[c]	N. E.
 **LPA**	0.83 ± 0.02 [100]	1.27 ± 0.5 [100]	3.36 ± 0.8 [100]

[a]For E_{max} > 30%, EC_{50} values are expressed as mean ± s.e.m, from a minimum of two independent experiments, performed in triplicate. [b]E_{max} = maximal efficacy of the drug/maximal efficacy of LPA, expressed as the percentage. [c]No effect was observed at the highest concentration of compound tested (10 μM).

*Biological characterization of compound **(S)-3a***

A set of additional experiments aimed at confirming that **(*S*)-3a** bound directly LPA_1 receptor and at studying whether the compound induced desensitization and internalization, were carried out.

The affinity of **(*S*)-3a** for the LPA_1 receptor was determined by backscattering interferometry. The obtained result (K_D = 19.9 nM) indicated that **(*S*)-3a** is a high affinity ligand with a K_D comparable to that obtained of the endogenous ligand LPA (K_D = 5.6 nM).

Then, it was demonstrated the capacity of compound **(*S*)-3a** to induce internalization of LPA_1 receptor. Representative images at 1 μM concentration of **(*S*)-3a** are shown in Figure 4.

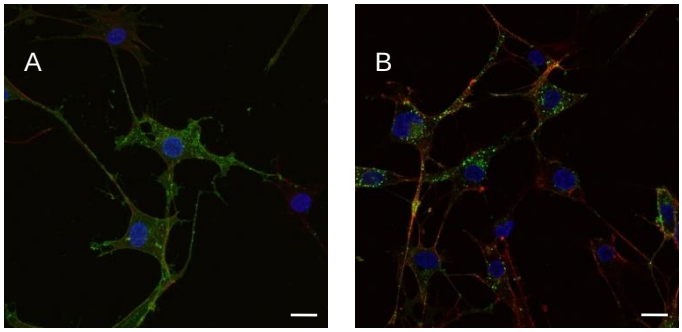


Figure 4. Receptor internalization of LPA_1 receptor by agonist stimulation. Cells were treated with compound **(*S*)-3a**, 1 μM (A) or LPA, 1 μM (B). Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and phalloydin for cell morphology. Samples were imaged under the same conditions by using a Zeiss fluorescence confocal microscope (bars 10 μm).

Finally, the capacity of **(*S*)-3a** to induce desensitization in sensory neurons was determined by measuring calcium influx and action potential. These experiments were

done in collaboration with Prof. Antonio Vicente Ferrer Montiel, at Universidad Miguel Hernández de Elche (Alicante, Spain). Our data show that after a first administration, compound **(*S*)-3a** activates the LPA_1 receptor in dorsal root ganglion (DRG) sensory neurons in primary culture in a similar manner to LPA (Figure 5). However, a second administration of the agonist 4 minutes later is not able to trigger activation, reflecting the desensitization of the receptor (Figure 5A). A similar behavior was observed for **(*S*)-3a** under the same conditions (Figure 5B). Furthermore, the action potential of DRG neurons was affected when treated either with 10 µM LPA (Figure 5C) or **(*S*)-3a** (Figure 5D), confirming the action of this agonist in sensory neurons in primary culture.

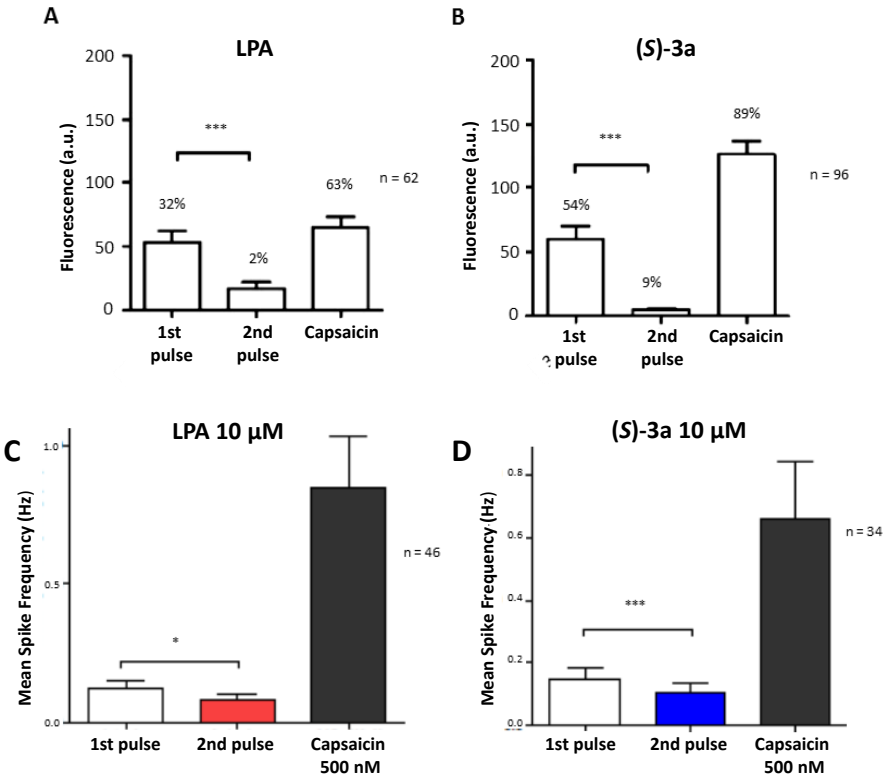


Figure 5. Measurement of calcium-influx peak intensity upon two repetitive applications of 10 µM LPA (A) or **(*S*)-3a** (B). Data represent fluorescence expressed as mean ± s.e.m. Effect of 10 µM LPA (C) or **(*S*)-3a** (D) on the neuronal firing activity on DRG neurons. LPA and **(*S*)-3a** were applied in two consecutive pulses (1st and 2nd pulse). Data represent mean spike frequency (Hz) and are expressed as mean ± s.e.m. Number of cultures ≥ 2. First pulse (1st) was compared to the second (2nd) by t-test paired values, ***p<0.001; *p<0.05. Number above columns represent percentage of responding cells to that stimulus, n= total registered neurons.

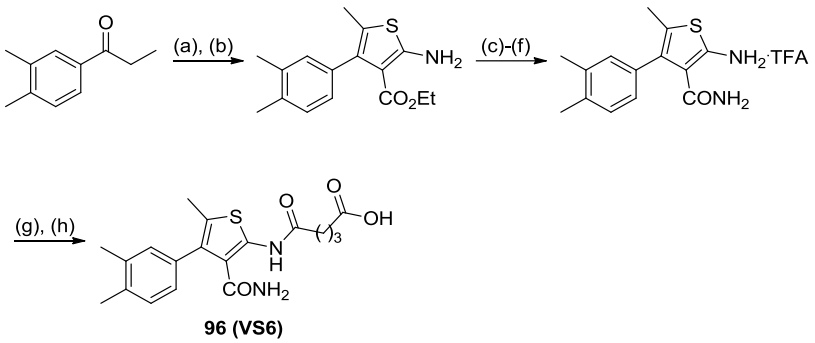
All in sum, the data obtained up to this moment have prompted us to consider **(*S*)-3a** a promising candidate for the treatment of NP and to study its in vivo efficacy in a mouse model of NP, experiments that are currently ongoing in collaboration with Prof. Fernando Rodríguez de Fonseca, at Instituto de Investigación Biomédica de Málaga (Spain).

5.2.2. Development of new antagonists for the LPA_1 and LPA_2 receptors

It has been recently described that activation of LPA_1 and LPA_2 receptors is detrimental for secondary damage after SCI, suggesting that LPA_1 and LPA_2 antagonists might be useful tools not only to elucidate the specific mechanisms implicated in this pathology, but also for its therapeutic treatment. In order to discover new antagonists at these receptors we performed a virtual screening (VS) of the ZINC library of compounds against the LPA_1 receptor, which 3D structure has just been described in complex with the selective antagonist ONO-9780307.[23]

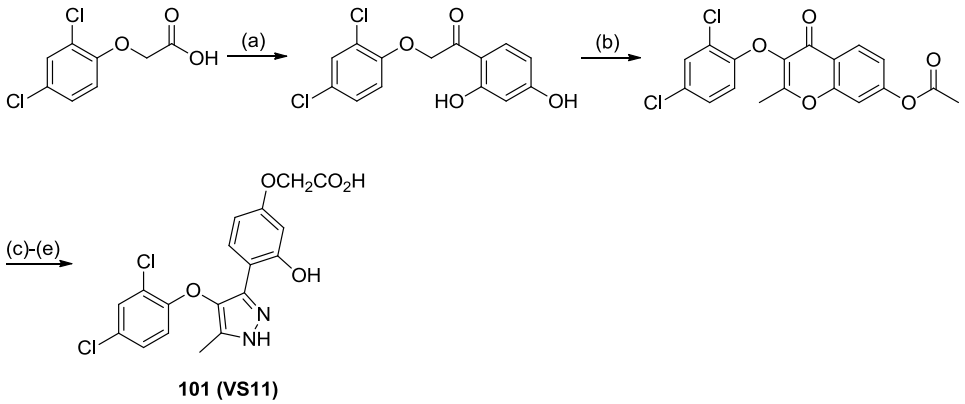
This VS yielded 18 potential hits, which were purchased and screened for antagonist activity at LPA_1, LPA_2 and LPA_3 receptors given their sequence similarity. Among all analyzed compounds, we were able to identify one compound selective for each receptor of interest, **VS6** (IC_{50} = 10.1 ± 0.9 µM) as LPA_1 receptor antagonist and **VS11** (IC_{50} = 1.9 ± 0.6 µM) as LPA_2 receptor antagonist.

Once the synthetic routes that allow to obtain both antagonists were set up (Schemes 7 and 8), their antagonist activity at LPA_{1-3} receptors was determined, confirming the half-maximum inhibitory concentration (IC_{50}) values obtained for the commercially supplied compounds **VS6** and **VS11**.



96 (VS6)

Scheme 7. Reagents and conditions: (a) Ethyl 2-cyanoacetate, $TiCl_4$, Et_3N, THF, 0ºC to rt, 12 h; (b) S_8, Et_2NH, THF, rt, 12 h, 32%; (c) Boc_2O, DMAP, THF, rt, 12 h, 28%; (d) KOH, EtOH:H_2O, 60°C, 12 h, 89%; (e) 0.5 M NH_3 in dioxane, EDC, HOBt, DIPEA, DCM, rt, 13 h, 70%; (f) TFA, DCM, rt, 12 h, 99%; (g) glutaric anhydride, Et_3N, DMAP, DCM, reflux, 12 h, 33%; (h) Et_3N, H_2O, reflux, 15 min, 72%.



Scheme 8. Reagents and conditions: (a) i) $SOCl_2$, toluene, 110°C, 12 h, 99%; ii) resorcinol, $BF_3{\cdot}Et_2O$, DCM, reflux, 4 h, 20%; (b) acetic anhydride, Et_3N, NaOAc, 140°C, 2.5 h, 99%; (c) HCl, EtOH, reflux, 2 h, 99%; (d) methyl 2-bromoacetate, K_2CO_3, acetone, reflux, 3 h, 51%; (e) 65% $N_2H_4{\cdot}H_2O$, EtOH, reflux, 30 min, 99%.

Derivative **101** (**VS11**) has been selected in order to establish whether an LPA_2 selective antagonist is able to induce a beneficial effect in SCI, which would be very interesting, considering the limited knowledge about the role of LPA_2 receptor in this pathology. Towards this aim, we have started determining which structural characteristics are important for the antagonist activity of compound **101**. Hence, we studied whether the 2,4-dichlorophenoxy system, the 3-hydroxyphenoxyacetic fragment or the methyl group in position 5 of the pyrazole ring are essential for the antagonism (Figure 6).

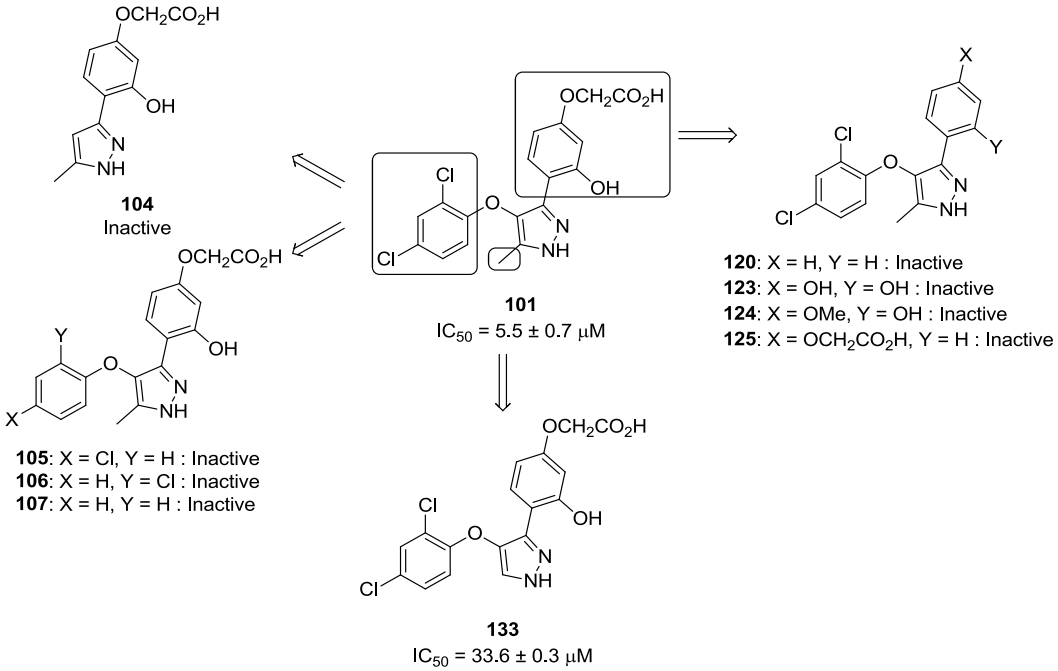


Figure 6. Study of the influence of the 2,4-dichlorophenoxy system, the 3-hydroxy phenoxyacetic fragment or the methyl group in the antagonist activity of compound **101**.

Once compounds **104**-**107**, **120**, **123**-**125**, and **133** were synthesized, they were tested for their antagonist activity at LPA_2 receptor. The obtained results indicated that the 2,4-dichlorophenoxy system and the 3-hydroxyphenoxyacetic fragment are essential for the antagonist activity, while the methyl group in position 5 of the pyrazole ring can be removed.

Before to start with a Medicinal Chemistry program with hit **101**, we have considered interesting, as proof of concept, demonstrate the interest of an LPA_2 selective antagonist in treatment of SCI. Towards this aim, we have started the first experiments needed to assess the suitability of antagonist **101** for these in vivo studies. Hence, this compound showed high binding affinity for LPA_2 receptor (K_D = 19.8 nM) and an acceptable cell permeability value (P = $1.2x10^{-6}$ cm/s), important features for subsequent in vivo studies, which are currently ongoing in collaboration with Prof. Rubèn Lopez Valés, at Universidad Autónoma de Barcelona (Spain).

Whether hit **101** is considered interesting, in a further work and as a continuation of this PhD thesis, an extended Medicinal Chemistry program with derivative **101** will be carried out, in order to obtain a second generation of antagonists at LPA_2 receptor.

5.3. Conclusions

In the present work we have addressed the development of new potent and selective LPA_1 receptor agonists, as antinociceptive agents, and of new selective antagonists of LPA_1 and LPA_2 receptors, as research tools to elucidate the specific involvement of these receptors in the pathophysiology of SCI.

With respect to the first goal, an exhaustive SAR study of several series of compounds, initially based on the endogenous ligand LPA, was performed and compound **(*S*)-3a** stood out for its high activity and affinity at LPA_1 receptor, and selectivity towards LPA_2 and LPA_3 receptors. In addition, this derivative promotes the internalization of LPA_1 receptor and its desensitization in sensory neurons, which should lead to the blockade of the signal trasduction involved in pain transmission. Hence, we are currently validating this approach as a possible therapeutic alternative to treat chronic pain in an in vivo model.

Regarding the second part of this work, a VS has allowed us to identify two potent and selective antagonists of LPA_1 and LPA_2 receptors. Once their synthetic routes were set up and their antagonist activities were confirmed, structural modifications of the LPA_2 receptor antagonist **101** (**VS11**) were carried out to determine which parts of the molecule are essential for its antagonist activity. Then, derivative **101** has been selected, as proof of concept, to start the study of the implication of this receptor in SCI.

5.4. Bibliography

1. Kihara, Y. *et al. Exp. Cell Res.* **2015**, *333*, 171-177.
2. Mutoh, T. *et al. Br. J. Pharmacol.* **2012**, *165*, 829-844.
3. Choi, J.W. *et al. Ann. Rev. Pharmacol. Toxicol.* **2010**, *50*, 157-186.
4. Yung, Y.C. *et al. J. Lipid Res.* **2014**, *55*, 1192-1214.
5. Lin, M.E. *et al. Prostaglandins Other Lipid Mediat.* **2010**, *91*, 130-138.
6. Choi, J.W. *et al. Biochim. Biophys. Acta* **2013**, *1831*, 20-32.
7. Frisca, F. *et al. Int. Rev. Cell Mol. Biol.* **2012**, *296*, 273-322.
8. Yung, Y.C. *et al. Neuron* **2015**, *85*, 669-682.
9. Velasco, M. *et al. Neuropharmacology* **2016**, *113*, 608-617.
10. Ma, L. *et al. Mol. Pain* **2013**, *9*, 1-14.
11. Inoue, M. *et al. Nat. Med.* **2004**, *10*, 712-718.
12. Ueda, H. *Biol. Pharm. Bull.* **2011**, *34*, 1154-1158.
13. Ueda, H. *et al. Biochim. Biophys. Acta* **2013**, *1831*, 61-73.
14. Santos-Nogueira, E. *et al. J. Neurosci.* **2015**, *35*, 10224-10235.
15. Santos Nogueria, E. PhD thesis, Universitat Autonoma de Barcelona, **2014**.
16. Goldshmit, Y. *et al. Am. J. Pathol.* **2012**, *181*, 978-992.
17. Nagai, J. *et al. Mol. Pain* **2010**, *6*, 3-11.
18. Goldshmit, Y. *et al. Cell Tissue Res.* **2010**, *341*, 23-32.
19. Murph, M.M. *et al. J. Cell Sci.* **2003**, *116*, 1969-1980.
20. Avendaño-Vázquez, S.E. *et al. Biochem. J.* **2005**, *385*, 677-684.
21. Alcántara-Hernández, R. *et al. PLoS One* **2015**, *10*, e0140583.
22. Burke, T.R. *et al. Accounts Chem. Res.* **2003**, *36*, 426-433.
23. Chrencik, J.E. *et al. Cell* **2015**, *161*, 1633-1643.

RESUMEN

6. RESUMEN

6.1. Introducción y Objetivos

Los lisofosfolípidos son derivados lípidicos con funciones tanto estructurales como de señalización, por lo que juegan un papel fundamental en humanos y otros mamíferos. El análisis de lisofosfolípidos en fluidos humanos de pacientes con diferentes condiciones patológicas revela no sólo la importancia de los lisofosfolípidos y de sus receptores en enfermedades humanas, sino también sus potenciales aplicaciones como biomarcadores y/o dianas terapéuticas.[1,2] Entre ellos, destaca el ácido lisofosfatídico (LPA) debido a su implicación en la regulación de procesos fisiológicos y patológicos de importancia a través de la activación de sus receptores correspondientes LPA_{1-6},[3-5] todos ellos pertenecientes a la superfamilia de los receptores acoplados a proteínas G (GPCRs).

El LPA regula una amplia variedad de actividades biológicas, especialmente en el desarrollo y el funcionamiento del sistema nervioso.[6,7] Considerando los numerosos efectos producidos por el LPA en diferentes tipos celulares del sistema nervioso y la sobreexpresión de los receptores de LPA como consecuencia de un daño neuronal observado tanto en ratones como en humanos, es probable que el LPA regule aspectos esenciales en la reorganización celular después de un trauma neuronal.[8] Entre todas las neuropatologías donde el LPA juega un papel importante, el dolor neuropático[9-13] y el daño medular[14-16] son condiciones con una elevada incidencia y altamente incapacitantes, que actualmente carecen de terapias específicas. En este contexto, los receptores LPA_1 y LPA_2 se han relacionado con estas patologías,[17,18] pero la falta de agonistas y antagonistas potentes y selectivos ha dificultado el estudio de sus funciones específicas. Por tanto, en este trabajo, hemos abordado el desarrollo de ambos tipos de compuestos para así tratar de esclarecer la función de los receptores LPA_1 y LPA_2 en el dolor neuropático y en el daño medular.

Este objetivo general implica las siguientes etapas:

1. Diseño y síntesis de nuevos ligandos con actividad agonista o antagonista en los receptores LPA_1 y LPA_2.
2. Determinación de la actividad y selectividad por los receptores de LPA.
3. Validación biológica de los compuestos seleccionados.

6.2. Resultados y Discusión

6.2.1. Desarrollo de nuevos agonistas del receptor LPA_1

Con respecto al dolor neuropático, hemos propuesto una estrategia basada en la unión de un agonista selectivo al receptor LPA_1 de tal modo que tras la activación receptorial se produce la desensibilización e internalización de éste, provocando así un efecto antinociceptivo de larga duración.[19-21]

La búsqueda de nuevas moléculas con actividad agonista por el receptor LPA_1 se basó en la modificación de la estructura del ligando endógeno, LPA. Para ello, se diseñaron inicialmente dos series de compuestos: la serie **I**, en la que se modifica el grupo ácido, y la serie **II**, que incluye modificaciones en la subunidad hidrofóbica (Figura 1).

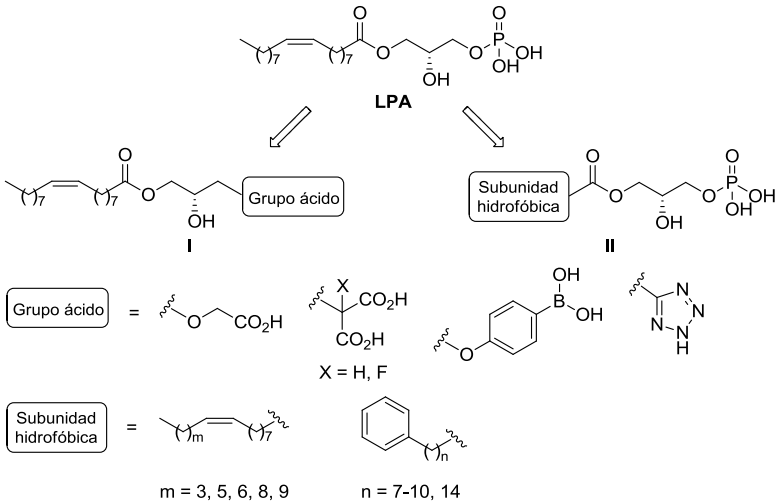
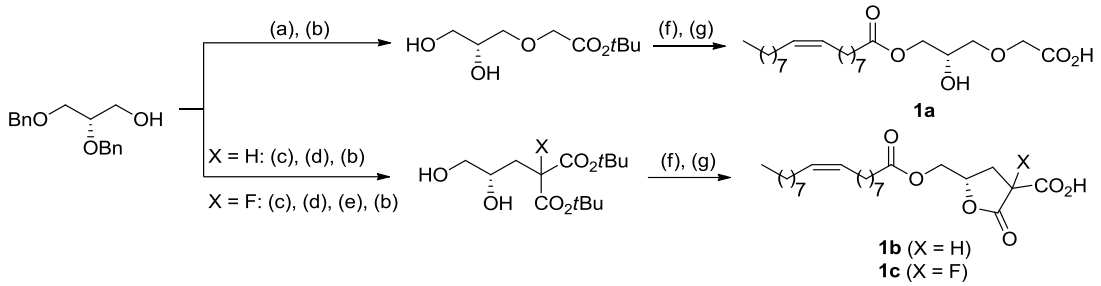
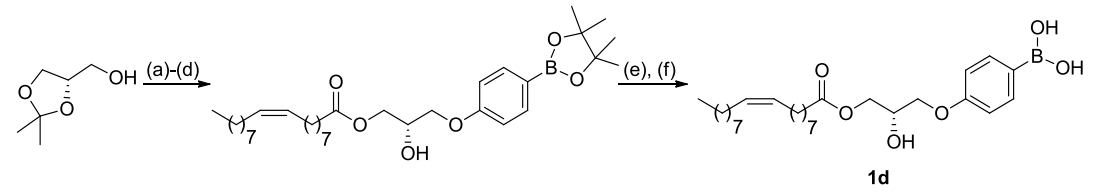


Figura 1. Diseño de nuevos ligandos agonistas del receptor LPA_1.

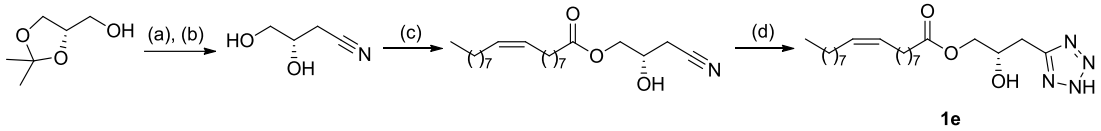
Con respecto a la serie **I**, se ha demostrado que modificaciones en el fragmento polar del LPA conllevan pérdida de actividad, por lo tanto, se eligió sustituir el grupo fosfato por otros fragmentos miméticos de éste, tales como ácidos carboxílicos y borónicos, o un anillo de tetrazol.[22] Los compuestos de la serie **I** fueron sintetizados como se indica en los Esquemas 1-3. En el caso de los ésteres malónicos, las condiciones de reacción empleadas condujeron a la formación de las lactonas **1b** y **1c** (Esquema 1).



Esquema 1. Reactivos y condiciones: (a) Bromoacetato de *tert*-butilo, NaH, TBAI, THF, 0ºC a 50ºC, 16 h, 22%; (b) H_2, Pd(C) 10%, EtOH, 60ºC, 89-95%; (c) cloruro de mesilo, Et_3N, DCM, 0ºC a ta, 1 h, 80%; (d) malonato de di-*tert*-butilo, NaH, NaI, DMF:THF, 0ºC a 80ºC, 17 h, 76%; (e) Selectfluor®, NaH, THF:DMF, 0ºC a ta, 48 h, 99%; (f) cloruro de oleoilo, 2,4,6-colidina, DCM, -78ºC a ta, 24 h, 36-99%; (g) TFA, DCM, ta, 17-18 h, 52-99%.



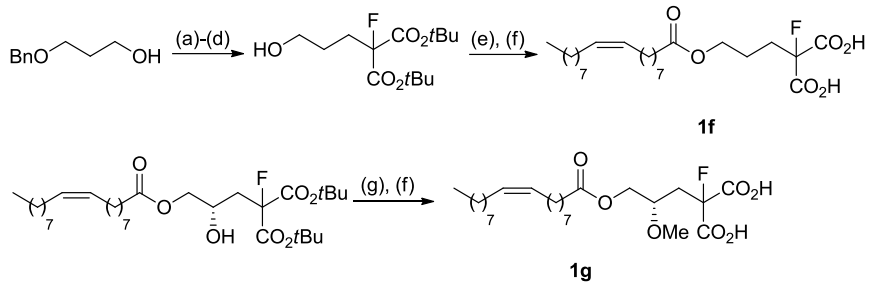
Esquema 2. Reactivos y condiciones: (a) Cloruro de tosilo, piridina, DCM, 0ºC a ta, 16 h, 86%; (b) 4-hidroxifenilpinacolborano, Cs_2CO_3, DMF, 90ºC, 16 h, 84%; (c) PS-*p*TsOH, CH_3OH, ta, 18 h, 88%; (d) cloruro de oleoilo, 2,4,6-colidina, DCM, -78ºC a ta, 24 h, 40%; (e) KHF_2, CH_3OH:H_2O, ta, 30 min; 99%; (f) TMSCl, CH_3CN:H_2O, ta, 1 h, 60%.



Esquema 3. Reactivos y condiciones: (a) i) Piridina, anhídrido tríflico, DCM, -20ºC, 30 min; ii) KCN, CH_3CN:H_2O, ta, 12 h, 99%; (b) TFA, CH_3OH, ta, 1.5 h, 61%; (c) cloruro de oleoilo, 2,4,6-colidina, DCM, -78ºC a ta, 12 h, 88%; (d) NaN_3, NH_4Cl, DMF, MW, 160ºC, 45 min, 5%.

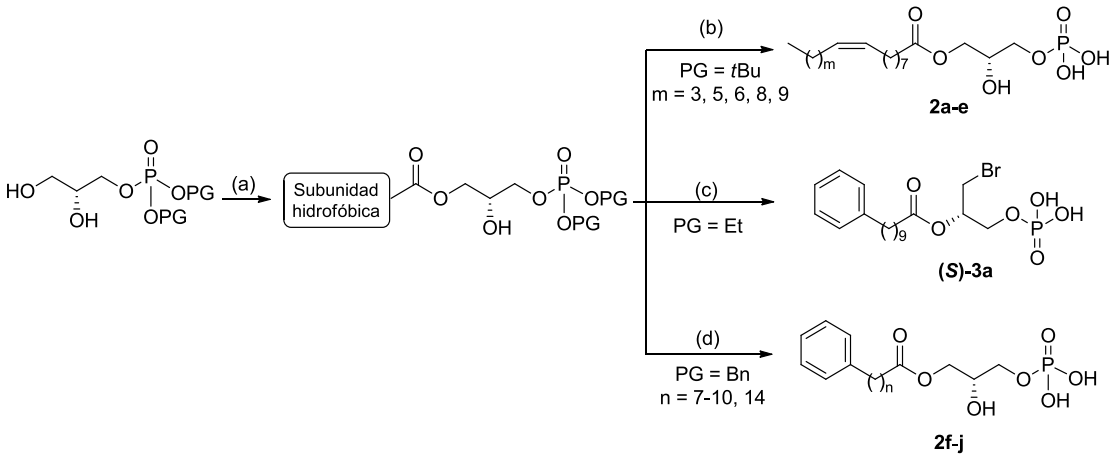
La capacidad de los compuestos sintetizados para activar el receptor LPA_1 se determinó mediante experimentos de movilización de calcio en células RH7777 transfectadas de forma estable con este receptor, puesto que su activación tanto por el LPA como por los ligandos agonistas produce un aumento en los niveles de calcio intracelular, el cual puede ser cuantificado mediante fluorescencia.

Así, el compuesto **1c** fue identificado como agonista del receptor LPA_1, con un valor de activación máxima del receptor (E_{max}) del 33% y un valor de concentración efectiva 50 (CE_{50}) de 1.7 µM. Por tanto, con objeto de obtener los derivados del ácido malónico propuestos inicialmente y evitar el proceso de ciclación intramolecular, se sintetizaron los compuestos **1f** y **1g** (Esquema 4). Entre ellos, únicamente el compuesto **1g** resultó activo, con un valor de CE_{50} de 6 µM.



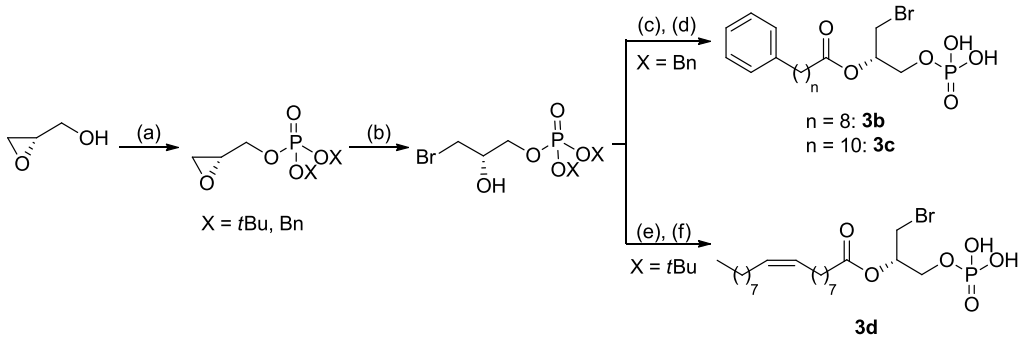
Esquema 4. Reactivos y condiciones: (a) Cloruro de mesilo, Et_3N, DCM, 0ºC a ta, 1 h, 99%; (b) malonato de di-*tert*-butilo, NaH, NaI, DMF:THF, 0ºC a 80ºC, 17 h, 66%; (c) Selectfluor®, NaH, THF:DMF, 0ºC a ta, 48 h, 99%; (d) H_2, Pd(C) 10%, EtOH, 60ºC, 95%; (e) cloruro de oleoilo, 2,4,6-colidina, DCM, -78ºC a ta, 24 h, 70%; (f) TFA, DCM, ta, 16-17 h, 90-95%; (g) trimetilsilildiazometano, HBF_4, DCM, 0ºC, 90 min, 38%.

En el caso de la serie **II**, se estudió exhaustivamente la influencia de la subunidad hidrofóbica, incluyendo cambios en la longitud total de la cadena de ácido graso, así como la incorporación de anillos aromáticos. La síntesis de los compuestos se representa en el Esquema 5.



Esquema 5. Reactivos y condiciones: (a) Ácido carboxílico, DCC, DMAP, DCM, -20ºC a ta, 16 h, 12-58%; (b) TFA, DCM, ta, 4-5 h, 90-99%; (c) i) TMSBr, DCM, ta, 4 h; ii) MeOH/H_2O, ta, 1h, 90%; (d) H_2, Pd(C) 10%, EtOH, ta, 80-99%.

Los compuestos **2a-j** y el producto inesperado **(*S*)-3a** fueron ensayados como agonistas del receptor LPA_1. Los datos obtenidos muestran la gran influencia que ejerce la longitud de la cadena hidrofóbica en la actividad. Entre todos los compuestos analizados, destacan los derivados **2b** (m = 5), **2h** (n = 9) y **(*S*)-3a**, con E_{max} > 100% y valores de CE_{50} de 0.45, 0.5 y 0.24 µM, respectivamente. Considerando la excelente actividad mostrada por el derivado **(*S*)-3a**, se decidió extender su estudio estructural (Esquema 6). Los resultados obtenidos indicaron que únicamente el derivado **3d** era capaz de activar el receptor (E_{max} = 39%; CE_{50} = 3.2 µM), aunque sin alcanzar los valores del compuesto **(*S*)-3a**.



Esquema 6. Reactivos y condiciones: (a) i) $(i\text{Pr})_2\text{NP(OX)}_2$, 1*H*-tetrazol, DCM, ta, 2 h; ii) *m*CPBA, -30ºC, 90 min, 52-76%; (b) TBABr, TFA, $CHCl_3$, ta, 10 min, 89-95%; (c) $\text{Ph(CH}_2)_n\text{COOH}$ (n = 8, 10), DCC, DMAP, DCM, ta, 16-18 h, 55-96%; (d) H_2, Pd(C) 10%, EtOH, ta, 72-86%; (e) cloruro de oleoilo, piridina, ta, 18 h, 15%; (f) TFA, DCM, ta, 5 h, 81%.

Selectividad de los agonistas del receptor LPA_1 frente a los receptores LPA_2 y LPA_3

Considerando la elevada similitud de secuencia entre los receptores LPA_{1-3}, hemos determinado la selectividad de los mejores agonistas de cada serie [**1c**, **1g**, **2b**, **2h** y **(*S*)-3a**]. La falta de actividad al receptor LPA_3 es especialmente importante, dado que su activación está implicada en la elevada producción de LPA que sigue a una lesión nerviosa periférica y que es responsable de la iniciación y el mantenimiento del dolor neuropático. Entre los agonistas analizados, los compuestos **1c**, **1g**, **2b** y **(*S*)-3a** resultaron selectivos por el receptor LPA_1 frente a los receptores LPA_2 y LPA_3 (Figura 2).

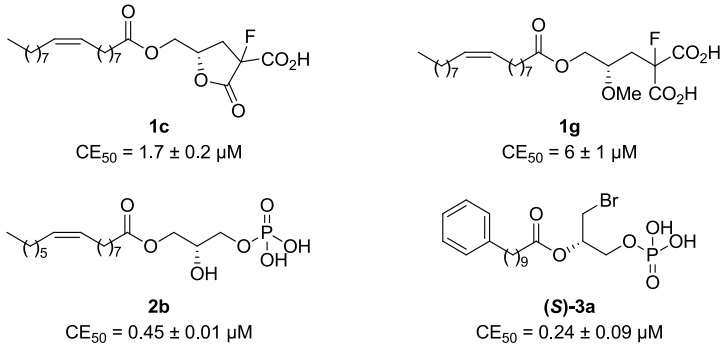


Figura 2. Agonistas del receptor LPA_1 con selectividad frente a los receptores LPA_2 y LPA_3.

Combinación de las subunidades ácidas e hidrofóbicas

A continuación, se combinaron las mejores subunidades hidrofóbicas y los grupos ácidos que resultaron ser capaces de mimetizar el grupo fosfato del LPA, manteniendo una potente actividad agonista y selectividad [compuestos **1c**, **1g**, **2b** y **(*S*)-3a**, Figura 2].

Por tanto, la lactona, el ácido malónico y el bromofosfato presentes en los derivados **1c**, **1g** y **(*S*)-3a**, respectivamente, fueron selecionados como grupos

ácidos. Como subunidades hidrofóbicas se eligieron las cadenas de oleoilo (presente en **1c** y **1g**), de palmitoleoilo (presente en **2b**) y de 10-fenildecanoilo [presente en **(*S*)-3a**]. La combinación de estos fragmentos condujo a los compuestos ya obtenidos con anterioridad [**1c**, **1g**, **(*S*)-3a** y **3d**] y a los nuevos productos **4a**-**e** (Figura 3) que fueron sintetizados y analizados como agonistas del receptor LPA_1. Entre los nuevos compuestos, únicamente el derivado **4c** presentó actividad por el receptor LPA_1 (E_{max} = 43 %, CE_{50} = 1.4 µM).

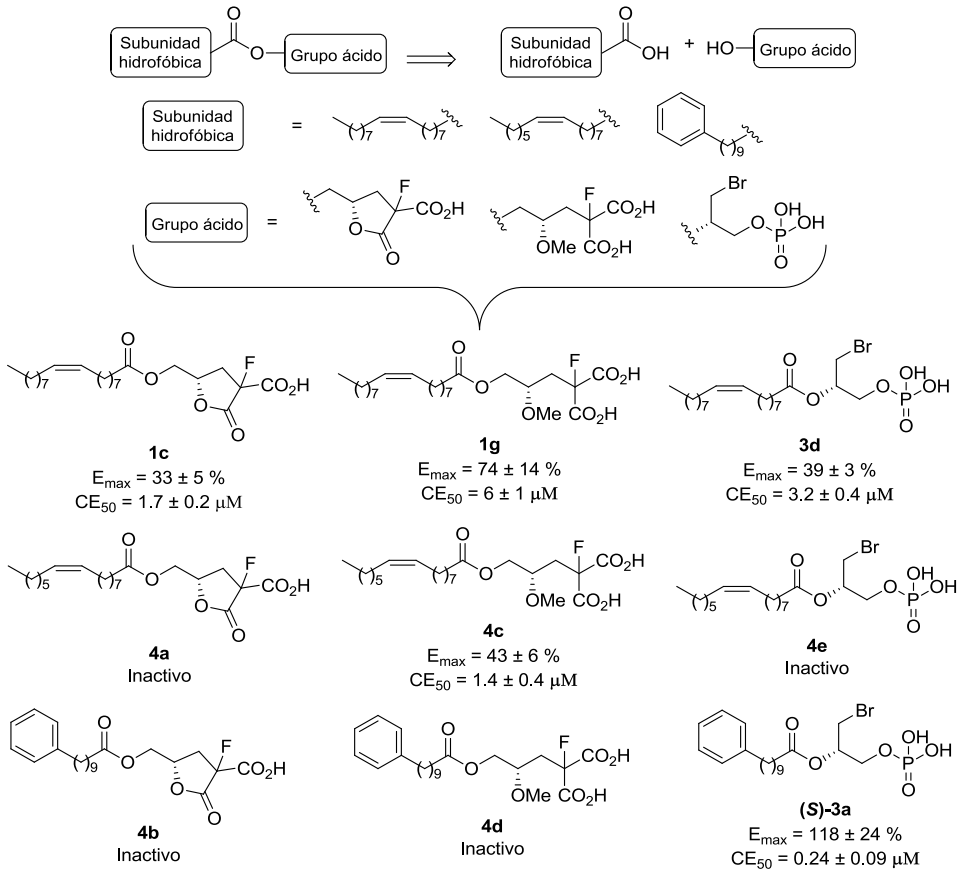
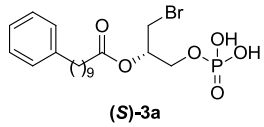
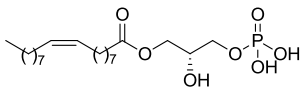


Figura 3. Combinación de las subunidades ácidas e hidrofóbicas de los compuestos óptimos.

Entre todos los compuestos sintetizados destaca el derivado **(*S*)-3a** como el primer agonista estructuralmente diferente al ligando endógeno LPA con excelentes valores de actividad y selectividad (Tabla 1).

Tabla 1. Actividad agonista del compuesto **(*S*)-3a** y del ligando endógeno LPA en los receptores LPA_{1-3}

Compuesto	CE_{50} (μM)[a] [E_{max} (%)][b]		
	Receptor LPA_1	**Receptor LPA_2**	**Receptor LPA_3**
 **(*S*)-3a**	0.24 ± 0.09 [118 ± 24]	N. E.[c]	N. E.
 **LPA**	0.83 ± 0.02 [100]	1.27 ± 0.5 [100]	3.36 ± 0.8 [100]

[a]Para E_{max} > 30%, los valores de CE_{50} corresponden al valor medio ± E.E. obtenido en un mínimo de dos experimentos independientes realizados por triplicado. [b]E_{max} = eficacia máxima del compuesto/eficacia máxima del LPA, expresado como porcentaje. [c]No se observa efecto a la máxima concentración ensayada (10 μM).

*Caracterización biológica del compuesto **(S)-3a***

Se realizaron diversos experimentos con el fin de confirmar la unión directa de **(*S*)-3a** al receptor LPA_1 así como su capacidad de inducir desensibilización e internalización del receptor LPA_1.

La afinidad de **(*S*)-3a** por el receptor LPA_1 fue determinada utilizando la técnica de *backscattering interferometry*. El resultado obtenido (K_D = 19.9 nM) indica que **(*S*)-3a** es un ligando de alta afinidad con un valor de K_D comparable al obtenido para el ligando endógeno LPA (K_D = 5.6 nM).

Asimismo, se ha demostrado la capacidad del compuesto **(*S*)-3a** para inducir la internalización del receptor LPA_1 (Figura 4).

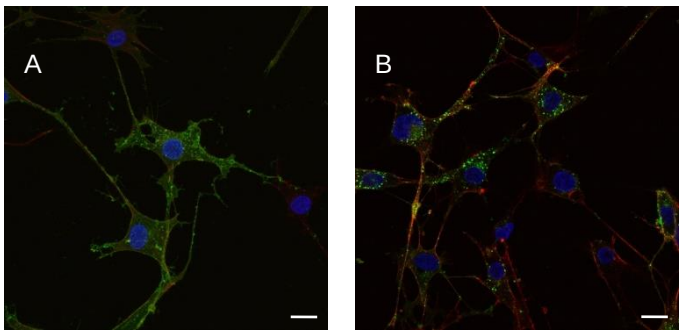


Figura 4. Internalización del receptor LPA_1 tras estimulación con un agonista. Las células fueron tratadas con el compuesto **(*S*)-3a** (1 μM) (A) o con LPA, 1 μM (B), y posteriormente teñidas con 4',6-diamino-2-fenilindol (DAPI) y faloidina para observar su morfología y visualizadas con un microscopio confocal Zeiss (barras 10 μm).

Por último, la capacidad del compuesto **(*S*)-3a** para inducir desensibilización en neuronas sensoriales fue determinada midiendo el flujo de calcio y el potencial de acción. Estos ensayos se han llevado a cabo en colaboración con el Prof. Antonio Vicente Ferrer Montiel, de la Universidad Miguel Hernández de Elche (Alicante). Los datos obtenidos muestran que una primera administración del compuesto **(*S*)-3a** conlleva la activación del receptor LPA_1 en neuronas sensoriales en cultivo primario de una forma parecida al LPA (Figura 5). Sin embargo, una segunda administración del agonista no es capaz de provocar activación, hecho que refleja la desensibilización del receptor (Figura 5A). Un comportamiento parecido se observó con el compuesto **(*S*)-3a** bajo las mismas condiciones (Figura 5B). Además, la activación de las neuronas también disminuye tras la segunda administración de LPA (Figura 5C) o de **(*S*)-3a** (Figura 5D), confirmando la actividad de estos agonistas en neuronas sensoriales.

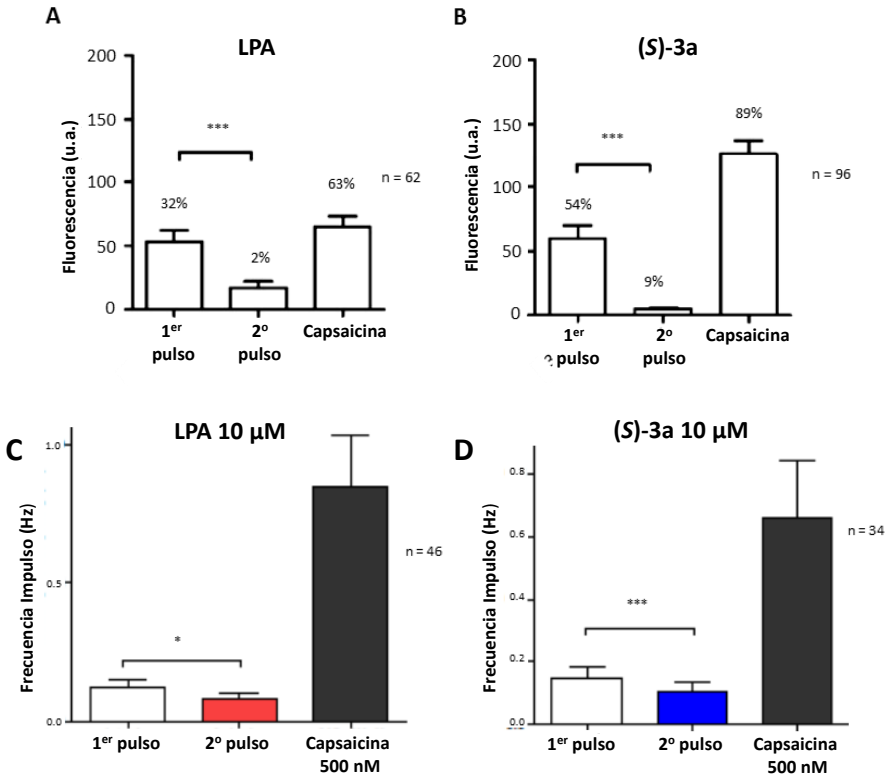


Figura 5. Aumento de los niveles de calcio tras dos aplicaciones repetidas de LPA (A) o **(*S*)-3a** (B). Los datos de fluorescencia corresponden al valor medio ± E.E. Efecto de LPA (C) o **(*S*)-3a** (D) en la actividad neuronal de neuronas de la raíz del ganglio dorsal. LPA y **(*S*)-3a** (ambos a una concentración de 10 µM) fueron aplicados en dos pulsos consecutivos. Los datos representan el valor de frecuencia (Hz) y corresponden al valor medio ± E.E. El número de cultivos es ≥ 2. El primer pulso fue comparado con el segundo utilizando la prueba t de Student, ***p<0.001; *p<0.05. Los números sobre las columnas representan el porcentaje de células que responden a este estímulo, donde n corresponde al número total de neuronas registradas.

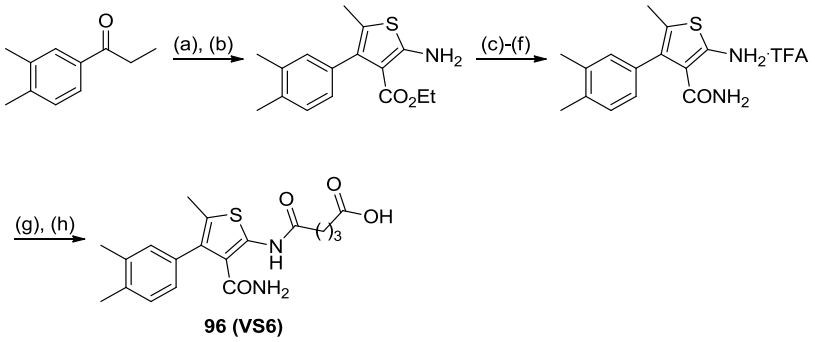
En resumen, los resultados obtenidos hasta el momento indican que el compuesto **(*S*)-3a** puede ser considerado como un candidato prometedor para el tratamiento del dolor neuropático y para estudiar su eficacia in vivo en un modelo de ratón de dolor neuropático, experimentos que se están actualmente llevando a cabo en colaboración con el Prof. Fernando Rodríguez de Fonseca, del Instituto de Investigación Biomédica de Málaga.

6.2.2. Desarrollo de nuevos antagonistas de los receptores LPA_1 y LPA_2

Recientemente se ha demostrado que la activación de los receptores LPA_1 y LPA_2 es perjudicial para el daño secundario que sigue a una lesión medular, sugiriendo que los antagonistas de los receptores de LPA_1 y LPA_2 podrían ser herramientas útiles no solo para esclarecer los mecanismos implicados en esta patología, sino también para su tratamiento. Para obtener nuevos antagonistas de estos receptores hemos llevado a cabo un cribado virtual de la librería ZINC de compuestos sobre el receptor LPA_1, cuya estructura cristalográfica ha sido descrita unida al antagonista selectivo ONO-9780307.[23]

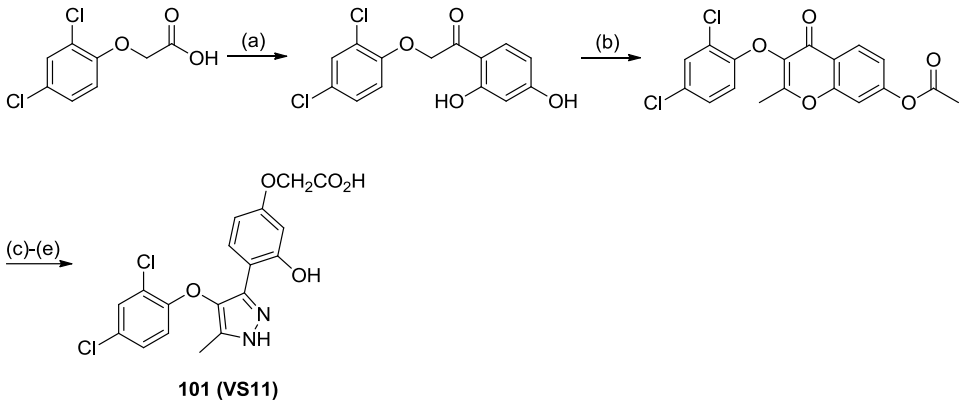
En el cribado virtual se identificaron 18 *hits*, los cuales fueron comprados y ensayados como antagonistas de los receptores LPA_1, LPA_2 y LPA_3. Entre todos los compuestos analizados, dos de ellos resultaron selectivos por los receptores de interés, **VS6** (CI_{50} = 10.1 ± 0.9 µM) como antagonista del receptor LPA_1 y **VS11** (CI_{50} = 1.9 ± 0.6 µM) como antagonista del receptor LPA_2.

Tras poner a punto las rutas sintéticas para obtener ambos antagonistas (Esquemas 7 y 8), se determinó su actividad en los receptores LPA_{1-3}, confirmando los valores de concentración inhibitoria 50 (CI_{50}) obtenidos para los compuestos comerciales **VS6** y **VS11**.



96 (VS6)

Esquema 7. Reactivos y condiciones: (a) Cianoacetato de etilo, $TiCl_4$, Et_3N, THF, 0ºC a ta, 12 h; (b) S_8, Et_2NH, THF, ta, 12 h, 32%; (c) Boc_2O, DMAP, THF, ta, 12 h, 28%; (d) KOH, EtOH:H_2O, 60°C, 12 h, 89%; (e) 0.5 M NH_3 en dioxano, EDC, HOBt, DIPEA, DCM, ta, 13 h, 70%; (f) TFA, DCM, ta, 12 h, 99%; (g) anhídrido glutárico, Et_3N, DMAP, DCM, reflujo, 12 h, 33%; (h) Et_3N, H_2O, reflujo, 15 min, 72%.



Esquema 8. Reactivos y condiciones: (a) i) $SOCl_2$, tolueno, 110°C, 12 h 99%; ii) resorcinol, $BF_3{\cdot}Et_2O$, DCM, reflujo, 4 h, 20%; (b) anhídrido acético, Et_3N, NaOAc, 140°C, 2.5 h, 99%; (c) HCl, EtOH, reflujo, 2 h, 99%; (d) 2-bromoacetato de metilo, K_2CO_3, acetone, reflujo, 3 h, 51%; (e) $N_2H_4{\cdot}H_2O$ 65%, EtOH, reflujo, 30 min, 99%.

El derivado **101** (**VS11**) fue seleccionado para establecer si un antagonista selectivo del receptor LPA_2 es capaz de inducir un efecto beneficioso en el daño medular, resultado que sería de gran interés considerando la escasa información acerca del papel del receptor LPA_2 en esta patología. Con este objetivo, hemos comenzado determinando las características estructurales responsables de la actividad antagonista del compuesto **101**. Por tanto, hemos estudiado si el sistema de 2,4-diclorofenoxilo, el fragmento 3-hidroxifenoxiacético o el grupo metilo en posición 5 del anillo de pirazol son esenciales para la actividad antagonista (Figura 6).

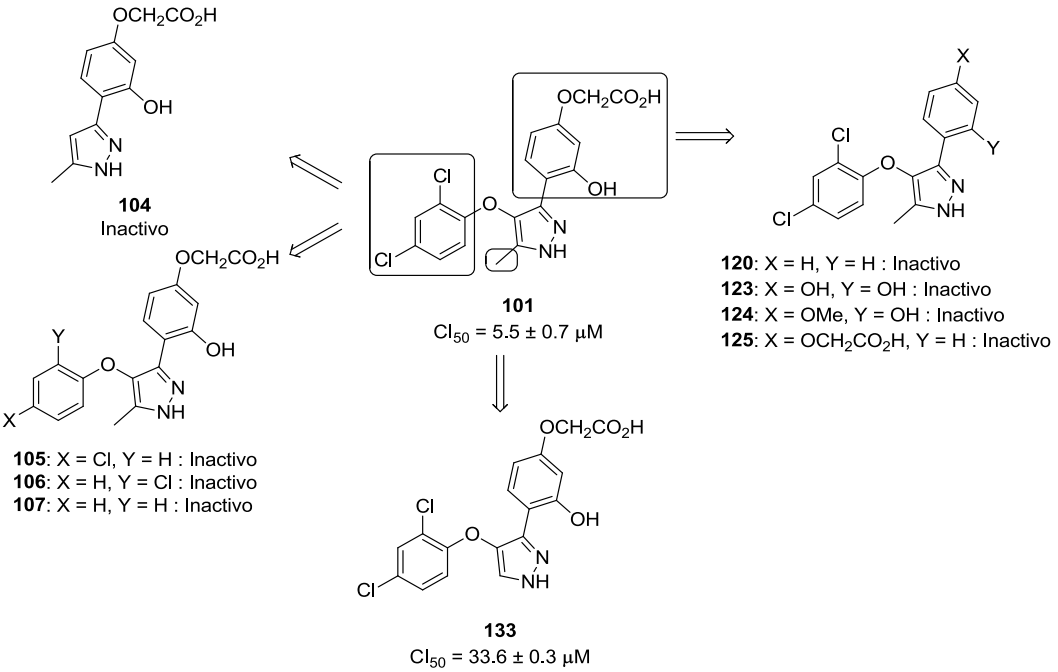


Figura 6. Estudio de la influencia del sistema de 2,4-diclorofenoxilo, del fragmento de 3-hidroxifenoxiacético o del grupo metilo en la actividad antagonista del compuesto **101**.

Una vez que los compuestos **104**-**107**, **120**, **123**-**125**, y **133** fueron sintetizados, se determinó su actividad antagonista en el receptor LPA_2. Los resultados obtenidos indicaron que los sistemas de 2,4-diclorofenoxilo y de 3-hidroxifenoxiacético son esenciales para la actividad antagonista, mientras que el grupo metilo en posición 5 del anillo de pirazol parece admitir modificaciones.

Antes de comenzar con un programa más extenso de Química Médica en el *hit* **101**, nos ha parecido interesante, como prueba de concepto, demostrar el interés de un antagonista del receptor LPA_2 en el tratamiento del daño medular. Con este objetivo, hemos iniciado los primeros experimentos previos a la evaluación in vivo. El compuesto **101** ha mostrado una elevada afinidad por el receptor LPA_2 (K_D = 19.8 nM) y un valor de permeabilidad celular aceptable ($P = 1.2 \times 10^{-6}$ cm/s), características necesarias para poder iniciar estudios in vivo, los cuales se están actualmente llevando a cabo en colaboración con el Prof. Rubèn Lopez Valés, de la Universidad Autónoma de Barcelona.

Si se confirma el interés del *hit* **101**, en un trabajo posterior y como continuación a esta Tesis Doctoral, se llevará a cabo un amplio programa de Química Médica a partir del compuesto **101**, con el objetivo de obtener antagonistas de segunda generación selectivos del receptor LPA_2.

6.3. Conclusiones

Durante el presente trabajo de investigación se han identificado nuevos agonistas potentes y selectivos del receptor LPA_1, y nuevos antagonistas selectivos de los receptores LPA_1 y LPA_2.

Dentro del primer objetivo, se ha llevado a cabo un estudio completo de relación estructura-actividad de diferentes series de compuestos, basados inicialmente en el ligando endógeno LPA. Entre los compuestos sintetizados, el derivado **(*S*)-3a** destacó por su alta actividad y afinidad por el receptor LPA_1, y selectividad frente a los receptores LPA_2 y LPA_3. Además, este agonista promueve la internalización del receptor LPA_1 y su desensibilización en neuronas sensoriales, lo que podría llevar a un bloqueo de la trasducción de la señal implicada en la transmisión del dolor. Por tanto, actualmente estamos validando esta estrategia como una posible alternativa terapéutica para el tratamineto del dolor crónico en un modelo in vivo.

Con respecto al segundo objetivo de este trabajo, un cribado virtual nos ha permitido identificar dos antagonistas potentes y selectivos de los receptores LPA_1 y LPA_2. Tras poner a punto las rutas sintéticas para su obtención y confirmar su actividad antagonista, se realizaron modificaciones estructurales del antagonista del receptor LPA_2 **101** (**VS11**) para determinar qué partes de la molecula son esenciales para su actividad antagonista. Por otro lado, el derivado **101** ha sido seleccionado para estudiar, como prueba de concepto, la implicación de este receptor en el daño medular.

6.4. Bibliografía

1. Kihara, Y. *et al. Exp. Cell Res.* **2015**, *333*, 171-177.
2. Mutoh, T. *et al. Br. J. Pharmacol.* **2012**, *165*, 829-844.
3. Choi, J.W. *et al. Ann. Rev. Pharmacol. Toxicol.* **2010**, *50*, 157-186.
4. Yung, Y.C. *et al. J. Lipid Res.* **2014**, *55*, 1192-1214.
5. Lin, M.E. *et al. Prostaglandins Other Lipid Mediat.* **2010**, *91*, 130-138.
6. Choi, J.W. *et al. Biochim. Biophys. Acta* **2013**, *1831*, 20-32.
7. Frisca, F. *et al. Int. Rev. Cell Mol. Biol.* **2012**, *296*, 273-322.
8. Yung, Y.C. *et al. Neuron* **2015**, *85*, 669-682.
9. Velasco, M. *et al. Neuropharmacology* **2016**, *113*, 608-617.
10. Ma, L. *et al*. *Mol. Pain* **2013**, *9*, 1-14.
11. Inoue, M. *et al. Nat. Med.* **2004**, *10*, 712-718.
12. Ueda, H. *Biol. Pharm. Bull.* **2011**, *34*, 1154-1158.
13. Ueda, H. *et al. Biochim. Biophys. Acta* **2013**, *1831*, 61-73.
14. Santos-Nogueira, E. *et al. J. Neurosci.* **2015**, *35*, 10224-10235.
15. Santos Nogueria, E. Tesis Doctoral, Universitat Autonoma de Barcelona, **2014**.
16. Goldshmit, Y. *et al. Am. J. Pathol.* **2012**, *181*, 978-992.

17. Nagai, J. *et al. Mol. Pain* **2010**, *6*, 3-11.
18. Goldshmit, Y. *et al. Cell Tissue Res.* **2010**, *341*, 23-32.
19. Murph, M.M. *et al. J. Cell Sci.* **2003**, *116*, 1969-1980.
20. Avendaño-Vázquez, S.E. *et al. Biochem. J.* **2005**, *385*, 677-684.
21. Alcántara-Hernández, R. *et al. PLoS One* **2015**, *10*, e0140583.
22. Burke, T.R. *et al. Accounts Chem. Res.* **2003**, *36*, 426-433.
23. Chrencik, J.E. *et al. Cell* **2015**, *161*, 1633-1643.

BIBLIOGRAPHY

7. BIBLIOGRAPHY

1. Kihara, Y.; Mizuno, H.; Chun, J. Lysophospholipid receptors in drug discovery. *Exp. Cell Res.* **2015**, *333*, 171-177.

2. Mutoh, T.; Rivera, R.; Chun, J. Insights into the pharmacological relevance of lysophospholipid receptors. *Br. J. Pharmacol.* **2012**, *165*, 829-844.

3. Bandoha, K.; Aokia, J.; Tairaa, A.; Tsujimotob, M.; Araia, H.; Inouea, K. Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species. *FEBS Lett.* **2000**, *478*, 159-165.

4. Pages, C.; Simon, M.F.; Valet, P.; Saulnier-Blache, J.S. Lysophosphatidic acid synthesis and release. *Prostaglandins Other Lipid Mediat.* **2001**, *64*, 1-10.

5. Aoki, J.; Inoue, A.; Okudaira, S. Two pathways for lysophosphatidic acid production. *Biochim. Biophys. Acta* **2008**, *1781*, 513-518.

6. Choi, J.W.; Herr, D.R.; Noguchi, K.; Yung, Y.C.; Lee, C.W.; Mutoh, T.; Lin, M.E.; Teo, S.T.; Park, K.E.; Mosley, A.N.; Chun, J. LPA receptors: subtypes and biological actions. *Ann. Rev. Pharmacol. Toxicol.* **2010**, *50*, 157-186.

7. Yung, Y.C.; Stoddard, N.C.; Chun, J. LPA receptor signaling: pharmacology, physiology, and pathophysiology. *J. Lipid Res.* **2014**, *55*, 1192-1214.

8. Fukushima, N.; Ishii, S.; Tsujiuchi, T.; Kagawa, N.; Katoh, K. Comparative analyses of lysophosphatidic acid receptor-mediated signaling. *Cell. Mol. Life Sci.* **2015**, *72*, 2377-2394.

9. Hecht, J.H.; Weiner, J.A.; Chun, J. *Ventricular zone gene-1 (vzg-1)* encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. *J. Cell Biol.* **1996**, *135*, 1071-1083.

10. González-Gil, I.; Zian, D.; Vázquez-Villa, H.; Ortega-Gutiérrez, S.; López-Rodríguez, M.L. The status of the lysophosphatidic acid receptor type 1 (LPA_1R). *Med. Chem. Commun.* **2015**, *6*, 13-23.

11. Contos, J.J.A.; Chun, J. Genomic characterization of the lysophosphatidic acid receptor gene, *lp$_{A2}$/Edg4*, and identification of a frameshift mutation in a previously characterized cDNA. *Genomics* **2000**, *64*, 155-169.

12. Bandoh, K.; Aoki, J.; Hosono, H.; Kobayashi, S.; Kobayashi, T.; Murakam-iMurofushi, K.; Tsujimoto, M.; Arai, H.; Inoue, K. Molecular cloning and characterization of a novel human G protein-coupled receptor, EDG7, for lysophosphatidic acid. *J. Biol. Chem.* **1999**, *274*, 27776-27785.

13. Lin, M.E.; Herr, D.R.; Chun, J. Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance. *Prostaglandins Other Lipid Mediat.* **2010**, *91*, 130-138.

14. Noguchi, K.; Ishii, S.; Shimizu, T. Identification of p2y$_9$/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. *J. Biol. Chem.* **2003**, *278*, 25600-25606.

15. Lee, C.W.; Rivera, R.; Dubin, A.E.; Chun, J. LPA$_4$/GPR23 is a lysophosphatidic acid (LPA) receptor utilizing G_s-, G_q/G_i-mediated calcium signaling and $G_{12/13}$-mediated Rho activation. *J. Biol. Chem.* **2007**, *282*, 4310-4317.

16. Lee, C.W.; Rivera, R.; Gardell, S.; Dubin, A.E.; Chun, J. GPR92 as a new $G_{12/13}$- and G_q-coupled lysophosphatidic acid receptor that increases cAMP, LPA$_5$. *J. Biol. Chem.* **2006**, *281*, 23589-23597.

17. Yanagida, K.; Masago, K.; Nakanishi, H.; Kihara, Y.; Hamano, F.; Tajima, Y.; Taguchi, R.; Shimizu, T.; Ishii, S. Identification and characterization of a novel lysophosphatidic acid receptor, p2y5/LPA$_6$. *J. Biol. Chem.* **2009**, *284*, 17731-17741.

18. McIntyre, T.M.; Pontsler, A.V.; Silva, A.R.; Hilaire, A.; Xu, Y.; Hinshaw, J.C.; Zimmerman, G.A.; Hama, K.; Aoki, J.; Arai, H.; Prestwich, G.D. Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARγ agonist. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 131-136.

19. Nieto-Posadas, A.; Picazo-Juárez, G.; Llorente, I.; Jara-Oseguera, A.; Morales-Lázaro, S.; Escalante-Alcalde, D.; Islas, L.D.; Rosenbaum, T. Lysophosphatidic acid directly activates TRPV1 through a C-terminal binding site. *Nat. Chem. Biol.* **2012**, *8*, 78-85.

20. Choi, J.W.; Chun, J. Lysophospholipids and their receptors in the central nervous system. *Biochim. Biophys. Acta* **2013**, *1831*, 20-32.

21. Frisca, F.; Sabbadini, R.A.; Goldshmit, Y.; Pebay, A. Biological effects of lysophosphatidic acid in the nervous system. *Int. Rev. Cell Mol. Biol.* **2012**, *296*, 273-322.

22. Yung, Y.C.; Stoddard, N.C.; Mirendil, H.; Chun, J. Lysophosphatidic acid signaling in the nervous system. *Neuron* **2015**, *85*, 669-682.

23. Gierthmühlen, J.; Baron, R. Neuropathic pain. *Semin. Neurol.* **2016**, *36*, 462-468.

24. Alba-Delgado, C.; Mico, J.A.; Sánchez-Blázquez, P.; Berrocoso, E. Analgesic antidepressants promote the responsiveness of locus coeruleus neurons to noxious stimulation: Implications for neuropathic pain. *Pain* **2012**, *153*, 1438-1449.

25. Doggrell, S.A. Pregabalin or morphine and gabapentin for neuropathic pain. *Expert Opin. Pharmacother.* **2005**, *6*, 2535-2539.

26. Ogawa, K.; Takasu, K.; Shinohara, S.; Yoneda, Y.; Kato, A. Pharmacological characterization of lysophosphatidic acid-induced pain with clinically relevant neuropathic pain drugs. *Eur. J. Pain* **2012**, *16*, 994-1004.

27. Ueda, H. Molecular mechanisms of neuropathic pain–phenotypic switch and initiation mechanisms. *Pharmacol. Ther.* **2006**, *109*, 57-77.

28. Velasco, M.; O'Sullivan, C.; Sheridan, G.K. Lysophosphatidic acid receptors (LPARs): Potential targets for the treatment of neuropathic pain. *Neuropharmacology* **2016**, *113*, 608-617.

29. McDougall, J.J.; Albacete, S.; Schuelert, N.; Mitchell, P.G.; Lin, C.; Oskins, J.L.; Bui, H.H.; Chambers, M.G. Lysophosphatidic acid provides a missing link between osteoarthritis and joint neuropathic pain. *Osteoarthritis Cartilage* **2016**.

30. Inoue, M.; Ma, L.; Aoki, J.; Chun, J.; Ueda, H. Autotaxin, a synthetic enzyme of lysophosphatidic acid (LPA), mediates the induction of nerve-injured neuropathic pain. *Mol. Pain* **2008**, *4*, 1-5.

31. Ma, L.; Uchida, H.; Nagai, J.; Inoue, M.; Aoki, J.; Ueda, H. Evidence for de novo synthesis of lysophosphatidic acid in the spinal cord through phospholipase A_2 and autotaxin in nerve injury-induced neuropathic pain. *J. Pharmacol. Exp. Ther.* **2010**, *333*, 540-546.

32. Ma, L.; Uchida, H.; Nagai, J.; Inoue, M.; Chun, J.; Aoki, J.; Ueda, H. Lysophosphatidic acid-3 receptor-mediated feed-forward production of lysophosphatidic acid: an initiator of nerve injury-induced neuropathic pain. *Mol. Pain* **2009**, *5*, 1-9.

33. Ma, L.; Nagai, J.; Ueda, H. Microglial activation mediates *de novo* lysophosphatidic acid poduction in a model of neuropathic pain. *J. Neurochem.* **2010**, *115*, 643-653.

34. Ma, L.; Nagai, J.; Chun, J.; Ueda, H. An LPA species (18:1 LPA) plays key roles in the self-amplification of spinal LPA production in the peripheral neuropathic pain model. *Mol. Pain* **2013**, *9*, 1-14.

35. Inoue, M.; Rashid, M.H.; Fujita, R.; Contos, J.J.A.; Chun, J.; Ueda, H. Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. *Nat. Med.* **2004**, *10*, 712-718.

36. Luo, Z.D.; Chaplan, S.R.; Higuera, E.S.; Sorkin, L.S.; Stauderman, K.A.; Williams, M.E.; Yaksh, T.L. Upregulation of dorsal root ganglion $\alpha_2\gamma$ calcium channel subunit

and its correlation with allodynia in spinal nerve-injured rats. *J. Neurosci.* **2001**, *21*, 1868-1875.

37. Ueda, H. Peripheral mechanisms of neuropathic pain–involvement of lysophosphatidic acid receptor-mediated demyelination. *Mol. Pain* **2008**, *4*, 1-13.

38. Nagai, J.; Uchida, H.; Matsushita, Y.; Yano, R.; Ueda, M.; Niwa, M.; Aoki, J.; Chun, J.; Ueda, H. Autotaxin and lysophosphatidic $acid_1$ receptor mediated demyelination of dorsal root fibers by sciatic nerve injury and intrathecal lysophosphatidylcholine. *Mol. Pain* **2010**, *6*, 3-11.

39. Ueda, H. Lysophosphatidic acid as the initiator of neuropathic pain. *Biol. Pharm. Bull.* **2011**, *34*, 1154-1158.

40. Morales-Lázaro, S.L.; Rosenbaum, T. A painful link between the TRPV1 channel and lysophosphatidic acid. *Life Sci.* **2015**, *125*, 15-24.

41. Xie, W.; Uchida, H.; Nagai, J.; Ueda, M.; Chun, J.; Ueda, H. Calpain-mediated down-regulation of myelin-associated glycoprotein in lysophosphatidic acid-induced neuropathic pain. *J. Neurochem.* **2010**, *113*, 1002-1011.

42. Parkinson, D.B.; Bhaskaran, A.; Arthur-Farraj, P.; Noon, L.A.; Woodhoo, A.; Lloyd, A.C.; Feltri, M.L.; Wrabetz, L.; Behrens, A.; Mirsky, R.; Jessen, K.R. c-Jun is a negative regulator of myelination. *J. Cell Biol.* **2008**, *181*, 625-637.

43. Ueda, H.; Matsunaga, H.; Olaposi, O.I.; Nagai, J. Lysophosphatidic acid: Chemical signature of neuropathic pain. *Biochim. Biophys. Acta* **2013**, *1831*, 61-73.

44. Sanchez, T.; Thangada, S.; Wu, M.T.; Kontos, C.D.; Wu, D.; Hong Wu, H.; Hla, T. PTEN as an effector in the signaling of antimigratory G protein-coupled receptor. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 4312-4317.

45. Li, Z.; Dong, X.; Wang, Z.; Liu, W.; Deng, N.; Ding, Y.; Tang, L.; Hla, T.; Zeng, R.; Li, L.; Wu, D. Regulation of PTEN by Rho small GTPases. *Nat. Cell Biol.* **2005**, *7*, 399-404.

46. Tsukahara, R.; Ueda, H. Myelin-related gene silencing mediated by LPA_1-Rho/ROCK signaling is correlated to acetylation of NFkB in S16 Schwann cells. *J. Pharmacol. Sci.* **2016**.

47. Saegusa, H.; Kurihara, T.; Zong, S.; Kazuno, A.; Matsuda, Y.; Nonaka, T.; Han, W.; Toriyama, H.; Tanabe, T. Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel. *Embo J.* **2001**, *20*, 2349-2356.

48. Uchida, H.; Matsumoto, M.; Ueda, H. Profiling of BoNT/C3-reversible gene expression induced by lysophosphatidic acid: ephrinB1 gene up-regulation underlying neuropathic hyperalgesia and allodynia. *Neurochem. Int.* **2009**, *54*, 215-221.

49. Caterina, M.J.; Schumacher, M.A.; Tominaga, M.; Rosen, T.A.; Levine, J.D.; Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **1997**, *389*, 816-824.

50. Caterina, M.J.; Leffer, A.; Malmberg, A.B.; Martin, W.J.; Trafton, J.; Petersen-Zeitz, K.R.; Koltzenburg, M.; Basbaum, A.I.; Julius, D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* **2000**, *288*, 306-313.

51. Tatsumi, S.; Mabuchi, T.; Katano, T.; Matsumura, S.; Abe, T.; Hidaka, H.; Suzuki, M.; Sasaki, Y.; Minami, T.; Ito, S. Involvement of Rho-kinase in inflammatory and neuropathic pain through phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS). *Neuroscience* **2005**, *131*, 491-498.

52. Ramer, L.M.; Borisoff, J.F.; Ramer, M.S. Rho-kinase inhibition enhances axonal plasticity and attenuates cold hyperalgesia after dorsal rhizotomy. *J. Neurosci.* **2004**, *24*, 10796-10805.

53. Gierse, J.; Thorarensen, A.; Beltey, K.; Bradshaw-Pierce, E.; Cortes-Burgos, L.; Hall, T.; Johnston, A.; Murphy, M.; Nemirovskiy, O.; Ogawa, S.; Pegg, L.; Pelc, M.; Prinsen, M.; Schnute, M.; Wendling, J.; Wene, S.; Weinberg, R.; Wittwer, A.; Zweifel, B.; Masferrer, J. A novel autotaxin inhibitor reduces lysophosphatidic acid levels in plasma and the site of inflammation. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 310-317.

54. Davies, S.P.; Reddy, H.; Caivano, M.; Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **2000**, *351*, 95-105.

55. Rowland, J.W.; Hawryluk, G.W.J.; Kwon, B.; Fehlings, M.G. Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. *Neurosurg. Focus* **2008**, *25*, 1-17.

56. Fehlings, M.G.; Theodore, N.; Harrop, J.; Maurais, G.; Kuntz, C.; Shaffrey, C.I.; Kwon, B.K.; Chapman, J.; Yee, A.; Tighe, A.; McKerracher, L. A phase I/IIa clinical trial of a recombinant Rho protein antagonist in acute spinal cord injury. *J. Neurotrauma* **2011**, *28*, 787-796.

57. Dennis, E.A.; Stephens, D.; David, S.; Lopez-Vales, R.; Kokotos, G.; Constantinou-Kokotou, V.; Barbayianni, E.; Magrioti, V. Phospholipase A_2 inhibitors and their use in treating neurological injury and disease. US 8420852 B2 **2013**.

58. Lopez-Vales, R.; Ghasemlou, N.; Redensek, A.; Kerr, B.J.; Barbayianni, E.; Antonopoulou, G.; Baskakis, C.; Rathore, K.I.; Constantinou-Kokotou, V.; Stephens, D.; Shimizu, T.; Dennis, E.A.; Kokotos, G.; David, S. Phospholipase A_2 superfamily members play divergent roles after spinal cord injury. *Faseb J.* **2011**, *25*, 4240-4252.

59. Antonopoulou, G.; Barbayianni, E.; Magrioti, V.; Cotton, N.; Stephens, D.; Constantinou-Kokotou, V.; Dennis, E.A.; Kokotos, G. Structure–activity relationships of natural and non-natural amino acid-based amide and 2-oxoamide inhibitors of human phospholipase A_2 enzymes. *Bioorg. Med. Chem.* **2008**, *16*, 10257-10269.

60. Dennis, E.; Kokotos, G.; Costantinou-Kokotou, V.; Davis, S. Amides as inhibitors of human secreted phospholipase A_2. WO 2010/011686 A2, **2010**.

61. Fournier, A.E.; Takizawa, B.T.; Strittmatter, S.M. Rho kinase inhibition enhances axonal regeneration in the injured CNS. *J. Neurosci.* **2003**, *23*, 1416-1423.

62. Goldshmit, Y.; Munro, K.; Leong, S.Y.; Pébay, A.; Turnley, A.M. LPA receptor expression in the central nervous system in health and following injury. *Cell Tissue Res.* **2010**, *341*, 23-32.

63. Santos Nogueria, E. PhD thesis, Spinal cord injury. Role of endothelial differentiation gene family lysophosphatidic acid receptors. Universitat Autonoma de Barcelona, **2014**.

64. Santos-Nogueira, E.; Lopez-Serrano, C.; Hernandez, J.; Lago, N.; Astudillo, A.M.; Balsinde, J.; Estivill-Torrus, G.; Rodriguez de Fonseca, F.; Chun, J.; Lopez-Vales, R. Activation of lysophosphatidic acid receptor type 1 contributes to pathophysiology of spinal cord injury. *J. Neurosci.* **2015**, *35*, 10224-10235.

65. Profyris, C.; Cheema, S.S.; Zang, D.W.; Azari, M.F.; Boyle, K.; Petratos, S. Degenerative and regenerative mechanisms governing spinal cord injury. *Neurobiol. Dis.* **2004**, *15*, 415-436.

66. Goldshmit, Y.; Matteo, R.; Sztal, T.; Ellett, F.; Frisca, F.; Moreno, K.; Crombie, D.; Lieschke, G.J.; Currie, P.D.; Sabbadini, R.A.; Pébay, A. Blockage of lysophosphatidic acid signaling improves spinal cord injury outcomes. *Am. J. Pathol.* **2012**, *181*, 978-992.

67. Crack, P.J.; Zhang, M.; Morganti-Kossmann, M.C.; Morris, A.J.; Wojciak, J.M.; Fleming, J.K.; Karve, I.; Wright, D.; Sashindranath, M.; Goldshmit, Y.; Conquest, A.; Daglas, M.; Johnston, L.A.; Medcalf, R.L.; Sabbadini, R.A.; Pébay, A. Anti-lysophosphatidic acid antibodies improve traumatic brain injury outcomes. *J. Neuroinflamm.* **2014**, *11*, 37.

68. Suardıaz, M.; Galan-Arriero, I.; Avila-Martin, G.; Estivill-Torrus, G.; de Fonseca, F.R.; Chun, J.; Gomez-Soriano, J.; Bravo-Esteban, E.; Taylor, J. Spinal cord compression injury in lysophosphatidic acid 1 receptor-null mice promotes maladaptive pronociceptive descending control. *Eur. J. Pain* **2016**, *20*, 176-185.

69. Ohta, H.; Sato, K.; Muata, N.; Damirin, A.; Malchinkhuu, E.; Kon, J.; Kimua, T.; Tobo, M.; Yamazaki, Y.; Watanabe, T.; Yagi, M.; Sato, M.; Suzuki, R.; Murooka, H.; Sakai, T.; Nishitoba, T.; Im, D.S.; Nochi, H.; Tamoto, K.; Tomura, H.; Okajima, F. Ki16425, a subtype-selective antagonist for EDG-family lysophosphatidic acid receptors. *Mol. Pharmacol.* **2003**, *64*, 994-1005.

70. Terakado, M.; Suzuki, H.; Hashimura, K.; Tanaka, M.; Ueda, H.; Kohno, H.; Fujimoto, T.; Saga, H.; Nakade, S.; Habashita, H.; Takaoka, Y.; Seko, T. Discovery of ONO-7300243 from a novel class of lysophosphatidic acid receptor 1 antagonists: from hit to lead. *ACS Med. Chem. Lett.* **2016**.

71. Swaney, J.S.; Chapman, C.; Correa, L.D.; Stebbins, K.J.; Bundey, R.A.; Prodanovich, P.C.; Fagan, P.; Baccei, C.S.; Santini, A.M.; Hutchinson, J.H.; Seiders, T.J.; Parr, T.A.; Prasit, P.; Evans, J.F.; Lorrain, D.S. A novel, orally active LPA_1 receptor antagonist inhibits lung fibrosis in the mouse bleomycin model. *Br. J. Pharmacol.* **2010**, *160*, 1699-1713.

72. Swaney, J.S.; Chapman, C.; Correa, L.D.; Stebbins, K.J.; Broadhead, A.R.; Bain, G.; Santini, A.M.; Darlington, J.; King, C.D.; Baccei, C.S.; Lee, C.; Parr, T.A.; Roppe, J.R.; Seiders, T.J.; Ziff, J.; Prasit, P.; Hutchinson, J.H.; Evans, J.F.; Lorrain, D.S. Pharmacokinetic and pharmacodynamic characterization of an oral lysophosphatidic acid type 1 receptor-selective antagonist. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 693-700.

73. Schaefer, M.; Perner-Storfer, J.; Kadere-It, D.; Strobel, H.; Czechtizky, W.; Chen, L.C. Acylamino-substituted fused cyclopentanecarboxylic acid derivatives and their use as pharmaceuticals. WO2009/135590A1, **2009**.

74. Santos-Nogueira, E.; Lopez-Vales, R. Methods for treating spinal cord injury with LPA receptor antagonists. WO 2013/070879 A1, **2013**.

75. Ferguson, S.S.G. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol. Rev.* **2001**, *53*, 1-24.

76. Gainetdinov, R.; Premont, R.T.; Bohn, L.M.; Lefkowitz, R.J.; Caron, M.G. Desensitization of G protein-coupled receptors and neuronal functions. *Annu. Rev. Neurosci.* **2004**, *27*, 107-144.

77. Kelly, E.; Bailey, C.P.; Henderson, G. Agonist-selective mechanisms of GPCR desensitization. *Br. J. Pharmacol.* **2008**, *153*, S379-S388.

78. Aktas, O.; Küry, P.; Kieseier, B.; Hartung, H.P. Fingolimod is a potential novel therapy for multiple sclerosis. *Nat. Rev. Neurol.* **2010**, *6*, 373-382.

79. Murph, M.M.; Scaccia, L.A.; Volpicelli, L.A.; Radhakrishna, H. Agonist-induced endocytosis of lysophosphatidic acid-coupled LPA_1/EDG-2 receptors via a dynamin2- and Rab5-dependent pathway. *J. Cell Sci.* **2003**, *116*, 1969-1980.

80. Avendaño-Vázquez, S.E.; Garcia-Caballero, A.; Garcia-Sáinz, J.A. Phosphorylation and desensitization of the lysophosphatidic acid receptor LPA_1. *Biochem. J.* **2005**, *385*, 677-684.

81. Alcántara-Hernández, R.; Hernández-Méndez, A.; Campos-Martínez, G.A.; Meizoso-Huesca, A.; García-Sáinz, J.A. Phosphorylation and internalization of lysophosphatidic acid receptors LPA_1, LPA_2, and LPA_3. *PLoS One* **2015**, *10*, e0140583.

82. Parrill, A.L.; Tigyi, G. Integrating the puzzle pieces: The current atomistic picture of phospholipid–G protein coupled receptor interactions. *Biochim. Biophys. Acta* **2013**, *1831*, 2-12.

83. Tigyi, G.; Parrill, A.L. Molecular mechanisms of lysophosphatidic acid action. *Prog. Lipid Res.* **2003**, *42*, 498-526.

84. Burke, T.R.; Lee, K. Phosphotyrosyl mimetics in the development of signal transduction inhibitors. *Accounts Chem. Res.* **2003**, *36*, 426-433.

85. Meanwell, N.A. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* **2011**, *54*, 2529-2591.

86. An, S.; Bleu, T.; Zheng, Y.; Goetzl, E.J. Recombinant human G protein-coupled lysophosphatidic acid receptors mediate intracellular calcium mobilization. *Mol. Pharmacol.* **1998**, *54*, 881-888.

87. Hopper, D.W.; Ragan, S.P.; Hooks, S.B.; Lynch, K.R.; Macdonald, T.L. Structure-activity relationships of lysophosphatidic acid: conformationally restricted backbone mimetics. *J. Med. Chem.* **1999**, *42*, 963-970.

88. Eckhardt, M.; Fu, G.C. The first applications of carbene ligands in cross-couplings of alkyl electrophiles: Sonogashira reactions of unactivated alkyl bromides and iodides. *J. Am. Chem. Soc.* **2003**, *125*, 13642-13643.

89. Brown, C.A.; Ahuja, V.K. Catalytic hydrogenation. VI. Reaction of sodium borohydride with nickel salts in ethanol solution. P-2 Nickel, a highly convenient, new, selective hydrogenation catalyst with great sensitivity to substrate structure. *J. Org. Chem.* **1973**, *38*, 2226-2230.

90. Orcajo-Rincon, A.L.; Ortega-Gutierrez, S.; Serrano, P.; Torrecillas, I.R.; Wuthrich, K.; Campillo, M.; Pardo, L.; Viso, A.; Benhamu, B.; Lopez-Rodriguez, M.L. Development of non-peptide ligands of growth factor receptor-bound protein 2-SRC homology 2 domain using molecular modeling and NMR spectroscopy. *J. Med. Chem.* **2011**, *54*, 1096-1100.

91. Cisneros, J.A.; Bjorklund, E.; Gonzalez-Gil, I.; Hu, Y.; Canales, A.; Medrano, F.J.; Romero, A.; Ortega-Gutierrez, S.; Fowler, C.J.; Lopez-Rodriguez, M.L. Structure-activity relationship of a new series of reversible dual monoacylglycerol lipase/fatty acid amide hydrolase inhibitors. *J. Med. Chem.* **2012**, *55*, 824-836.

92. Durgam, G.G.; Virag, T.; Walker, M.D.; Tsukahara, R.; Yasuda, S.; Liliom, K.; van Meeteren, L.A.; Moolenaar, W.H.; Wilke, N.; Siess, W.; Tigyi, G.; Miller, D.D. Synthesis, structure-activity relationships, and biological evaluation of fatty alcohol phosphates as lysophosphatidic acid receptor ligands, activators of PPARgamma, and inhibitors of autotaxin. *J. Med. Chem.* **2005**, *48*, 4919-4930.

93. Jiang, G.; Xu, Y.; Fujiwara, Y.; Tsukahara, T.; Tsukahara, R.; Gajewiak, J.; Tigyi, G.; Prestwich, G.D. Alpha-substituted phosphonate analogues of lysophosphatidic acid (LPA) selectively inhibit production and action of LPA. *ChemMedChem* **2007**, *2*, 679-690.

94. Plueckthun, A.; Dennis, E.A. Acyl and phosphoryl migration in lysophospholipids: importance in phospholipid synthesis and phospholipase specificity. *Biochemistry* **1982**, *21*, 1743-1750.

95. Fukushima, N.; Kimura, Y.; Chun, J. A single receptor encoded by vzg-1 / lpA1 / edg-2 couples to G proteins and mediates multiple cellular responses to lysophosphatidic acid. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6151-6156.

96. Righi, G.; Ciambrone, S. Stereocontrolled addition of boron enolates to *trans* α,β-aziridine aldehydes. A new route to *anti*-1,2-amino alcohols. *Tetrahedron Lett.* **2004**, *45*, 2103-2106.

97. Baksh, M.M.; Kussrow, A.K.; Mileni, M.; Finn, M.G.; Bornhop, D.J. Label-free quantification of membrane-ligand interactions using backscattering interferometry. *Nat. Biotechnol.* **2011**, *29*, 357-360.

98. Bornhop, D.J.; Latham, J.C.; Kussrow, A.; Markov, D.A.; Jones, R.D.; Sørensen, H.S. Free-solution, label-free molecular interactions studied by back-scattering interferometry. *Science* **2007**, *317*, 1732-1736.

99. Budd, D.C.; Qian, Y. Development of lysophosphatidic acid pathway modulators as therapies for fibrosis. *Future Med. Chem.* **2013**, *5*, 1935-1952.

100. Parrill, A.L. Design of anticancer lysophosphatidic acid agonists and antagonists. *Future Med. Chem.* **2014**, *6*, 871-883.

101. Llona-Minguez, S.; Ghassemian, A.; Helleday, T. Lysophosphatidic acid receptor (LPAR) modulators: The current pharmacological toolbox. *Prog. Lipid Res.* **2015**, *58*, 51-75.

102. Chrencik, J.E.; Roth, C.B.; Terakado, M.; Kurata, H.; Omi, R.; Kihara, Y.; Warshaviak, D.; Nakade, S.; Asmar-Rovira, G.; Mileni, M.; Mizuno, H.; Griffith, M.T.; Rodgers, C.; Han, G.W.; Velasquez, J.; Chun, J.; Stevens, R.C.; Hanson, M.A. Crystal structure of antagonist bound human lysophosphatidic acid receptor 1. *Cell* **2015**, *161*, 1633-1643.

103. Wang, W.; Lv, D.; Qiu, N.; Zhang, L.; Hu, C.; Hu, Y. Design, synthesis and biological evaluation of novel 3,4,5-trisubstituted aminothiophenes as inhibitors of p53–MDM2 interaction. Part 2. *Bioorg. Med. Chem.* **2013**, *21*, 2886-2894.

104. Bundesmann, M.W.; Coffey, S.B.; Wright, S.W. Amidation of esters assisted by $Mg(OCH_3)_2$ or $CaCl_2$. *Tetrahedron Lett.* **2010**, *51*, 3879-3882.

105. Hadida-Ruah, S.; Hamilton, M.; Miller, M.; Grootenhuis, P.D.J.; Bear, B.; McCartney, J.; Zhou, J.; Van Goor, F. Modulators of ATP-binding cassette transporters. US2008019915, **2008**.

106. Savariar, E.N.; Aathimanikandan, S.V.; Thayumanavan, S. Supramolecular assemblies from amphiphilic homopolymers: testing the scope. *J. Am. Chem. Soc.* **2006**, *128*, 16224-16230.

107. Iwasaki, T.; Higashikawa, K.; Reddy, V.P.; Ho, W.W.S.; Fujimoto, Y.; Fukase, K.; Terao, J.; Kuniyasu, H.; Kambe, N. Nickel-butadiene catalytic system for the cross-coupling of bromoalkanoic acids with alkyl grignard reagents: a practical and versatile method for preparing fatty acids. *Chemistry - A European Journal* **2013**, *19*, 2956-2960.

108. Jeon, O.Y.; Carreira, E.M. Differential interaction of an AmB analogue and ergosterol in enantiomeric membranes. *Org. Lett.* **2010**, *12*, 1772-1775.

109. Williams, L.; Zhang, Z.; Shao, F.; Carroll, P.J.; Joulli, M.M. Grignard reactions to chiral oxazolidine aldehydes *Tetrahedron* **1996**, *52*, 11673-11694.

110. Boibessot, T.; Bénimèlis, D.; Jean, M.; Benfodda, Z.; Meffre, P. Synthesis of a novel rhizobitoxine-like triazole-containing amino acid. *Synlett* **2016**, *27*, 2685-2688.